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THOMPSON YATES LABORATORIES
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THE THOMPSON YATES LABORATORIES REPORT

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CONTENTS

Report of the Liverpool Expedition to Nigeria—Part II.	Filariasis	1
	<i>H. E. Annett</i>	
	<i>J. Everett Dutton</i>	
	<i>J. H. Elliott</i>	
A Preliminary Note on the Hibernation of Mosquitoes .	<i>H. E. Annett</i>	93
	<i>J. Everett Dutton</i>	
The Flora of the Conjunctiva in Health and Disease .	<i>A. S. Griffith</i>	99
I. A Proposed Simple Test for Faecal Contamination ; and II. The Behaviour in Taurocholate-glucose-litmus Broth, in Certain Sugars, and in Glycerine, of some of the Commoner Organisms—with Special Reference to the Effect of their Presence upon the Value of the above Test	<i>Alfred MacConkey</i>	151
	<i>Charles A. Hill</i>	
Milk as a Vehicle of Tubercle and Present Local Legislation in Regard to it		169
	<i>E. W. Hope</i>	
The Excretory and Tubercular Contamination of Milk	<i>Rubert Boyce</i>	177
Report of the Bacteriological Investigations and Analyses made for the Corporation of Liverpool	<i>Rubert Boyce</i>	183
Note on Pink Eye in Horses	<i>C. Balfour Stewart</i>	203
	<i>Rubert Boyce</i>	
Report of the Librarian		209

REPORT OF THE
MALARIA EXPEDITION TO NIGERIA

PART II. FILARIASIS

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LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR IV

REPORT
OF THE
MALARIA EXPEDITION TO
NIGERIA

OF THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

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PART II. FILARIASIS

WITH ILLUSTRATIONS AND PLATES

AT THE UNIVERSITY PRESS OF LIVERPOOL 1901

ISSUED BY THE COMMITTEE

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

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PREFACE

The series of new blood filariae described in the following pages were found during the examination of a large number of West African birds of different species for parasites of the red blood corpuscles. The discovery of the blood filariae naturally led to a search for their parent forms: but time did not permit of any extensive investigations being made as to the nature of their intermediary hosts.

Opportunities also occurred for observations on human filariasis in West Africa, which combined with the work on avian filariasis, will, it is hoped, throw considerable light on this very interesting branch of parasitology.

The description of the parasites has involved a great amount of labour in the examination of the literature of the subject, and for this reason, and also because of the rapidly increasing importance of the subject, and of the desire for a comprehensive work, often expressed by investigators in tropical countries, it has been considered desirable to incorporate in this work STROSSICH's extensive bibliography, and also to introduce chapters on the Nematodes in general and the Filariae in particular, for the greater part of the matter of which we are indebted to the valuable works of SHIPLEY (*Worms*, etc., The Cambridge Natural History) and RAILLIET (*Zoologie médicale et agricole*).

The authors wish particularly to thank Mr. A. E. SHIPLEY for his useful advice and help; Mr. ROBINSON, who kindly undertook the identification of the birds of our collection; Dr. A. H. HANLEY for much valuable material; and our colleagues at University College for their assistance.

H. E. A.

J. E. D.

J. H. E.

September, 1901

CONTENTS

	PAGE
<i>Chapter I.</i> INTRODUCTION	I
<i>Chapter II.</i> THE FILARIAE	11
<i>Chapter III.</i> AVIAN FILARIAE	23
<i>Chapter IV.</i> HUMAN FILARIASIS	43
FILARIA NOCTURNA, DIURNA, AND PERSTANS	
Geographical Distribution	52
Observations on Distribution in West Africa	54
PERIODICITY.	57
INTERMEDIARY HOST OF <i>F. nocturna</i>	69
FILARIAE IN <i>Anopheles costalis</i>	72
THE ANATOMY OF THE MOUTH PARTS OF THE FEMALE	
<i>Anopheles costalis</i>	73
THE RELATION BETWEEN <i>F. nocturna</i> AND <i>F. diurna</i>	89

APPENDIX.

DESCRIPTION OF PLATES TO APPENDIX.

PLATES TO APPENDIX.

DESCRIPTION OF PLATES.

PLATES.

BIBLIOGRAPHY.

REPORT OF LIVERPOOL EXPEDITION TO NIGERIA

PART II

I. *FILARIASIS*

INTRODUCTION

THE NEMATHELMINTHES, the order to which the genus *Filaria* belongs, have the following characteristics :—they are worm-like in form, but non-segmented ; that is, their bodies are not divided into segments, each resembling more or less exactly in outward appearance and internal structure the preceding and following segment. Many bear bristles or hooks, and exceptionally suckers. The body is elongated, thread-like, enclosed in a more or less thick cuticle. They have no closed vascular system nor special respiratory organs. They are almost all dioecious—the male and female reproductive organs being in different individuals. The young somewhat resemble the adults, but have no sexual organs ; the immature stages, termed *larvae*, are often free while the adults are parasitic or *vice versâ*, or inhabit a different host from the adult. Some of these Nematelminthes spend their life within the bodies of their hosts, or are only parasitic during a portion ; a few have a free life in water or damp earth.

The Nematelminthes comprise three sub-orders :—

- 1.—The Nematoda
- 2.—The Nematomorpha (Gordiidae)
- 3.—The Acanthocephala

The Nematoda have a complete digestive tube ; in the Nematomorpha it is atrophied in the adult, while in the adults of Acanthocephala it is absent altogether. In the sub-order Nematomorpha (Gordiidae) are two genera, *Gordius* and *Nectonema* ; the latter has only a single species, *Nectonema agile*, which is marine. The genus *Gordius*, which is entirely fresh water, has a large number of species. Worms of this genus pass through three stages, two larval and parasitic, the third, sexually mature, living in water. The first larval stage has been found in the larvae of *Sialis lutaria*, *Ephemera*, *Tanytus*, *Corethra*, *Chironomus* ; the second is parasitic in the bodies of *Carabus hortensis*, *Procerus* (*Carabus*), *Coriaceus*, *Calathus fuscipes*, *Molops elatus*, several species of *Pterostichus*, and other beetles.

According to RAILLIET¹ cases have been recorded in which the adult forms of some species of this genus have been evacuated after the administration of an anthelmintic—in some of the cases troublesome symptoms occurred. The actual

1. Railliet, *Traité de Zoologie Médicale et Agricole*. Paris, 1895. P. 563.

species described as occurring thus, are :—*Gordius aquaticus*, *G. tolosanus*, *G. varius*, *G. chilensis*. Probably they gain access to the alimentary canal of man and animals through the medium of drinking water.

The Acanthacephala include, following SHIPLEY,² four families:—Neorhynchidae, Gigantorhynchidae, Echinorhynchidae, and Arhynchidae. The adult forms have no alimentary tract, and are provided with a retractable proboscis, armed with hooklets, arranged in longitudinal rows. The adult stage occurs in the alimentary canal of vertebrates, generally those which live in or near water; while the larvae are found in the bodies of certain invertebrates, generally small Crustacea³, e.g., *Gigantorhynchus gigas* inhabits the small intestine of the pig, wild boar, and occasionally man, while the larval host is believed to be some species of beetle (*Melolontha*, *Cetonia*, and *Lachnosterna*). *G. echinodiscus* inhabits the intestine of ant eaters; *G. spira* of the king vulture; *Echinorhynchus proteus* of fishes (gudgeon, trout, turbot, etc.); the larval stage in some Amphipod Crustacea, and some fresh water fishes (minnow, etc.). Other species of *Echinorhynchus* occur in the duck, dog, rabbit, some aquatic birds, and occasionally man.

The *Nematodes* present very great difficulties to the systematist in their classification. SCHNEIDER⁴ divided them into three groups:—(i) the Polymyarii, in which numerous muscle cells are seen in a transverse section; (ii) the Meromyarii, in which only eight are seen, two in each quadrant; (iii) the Holomyarii, in which the muscles are either not divided or only divided by longitudinal lines. Other classifications have been based upon the life history, but in many cases this is only very imperfectly known. At present the arrangement of the muscles (Polymyarii, Meromyarii, Holomyarii), the arrangement of the lips and mouth parts, the character of the male tail, the number of papillae, and the number and size of spicules, are the features which are relied upon for classification. SHIPLEY⁵ deems it advisable at present to abandon the larger groups, and to deal directly with families. Of these he quotes seven:—

- I. Ascaridae.
- II. Strongylidae.
- III. Trichotrachelidae.
- IV. Filariidae.
- V. Mermithidae.
- VI. Anguillulidae.
- VII. Enoplidae.

We have considered it advisable to state briefly here the characteristic features of each of these families, and to describe shortly those forms of each family which are interesting to the student of human parasitology.

2. Harmer and Shipley, *The Cambridge Natural History*, Vol. II, *Worms, Rotifers, and Polyzoa*. London, 1896. P. 182

3. Harmer and Shipley, *The Cambridge Natural History*, Vol. II, *Worms, Rotifers, and Polyzoa*. London, 1896. P. 174.

4. Schneider, *Monographie der Nematoden*. Berlin, 1866.

5. Harmer and Shipley, *The Cambridge Natural History*, Vol. II, *Worms*, etc. P. 138.

I. ASCARIDAE

SHIPLEY⁶ gives the following characteristics: 'Body rather stout. A dorsal and two ventro-lateral lips bearing papillae. Buccal cavity distinct, seldom provided with chitinous armature. The oesophagus has two dilatations. The tail of the male is ventrally curved, and usually there are two horny spicules.' The females have a double ovary, and are generally oviparous.

Genera: *Ascaris*, *Heterakis*, *Oxyuris*, *Nematoxys*, *Oxysoma*, and others.

Genus *Ascaris*. These are polymyarian and have three lips, generally bearing teeth. The males have two equal spicules and a number of pre- and post-anal papillae, by the latter of which the best specific characters are furnished. The vulva is situated about the middle of the body. The ova are globular or ellipsoidal. They inhabit the intestines of their respective hosts. The species are very numerous.

The *life history* has not been completely worked out. Infection experiments by feeding directly with material containing ova have always failed. It is probable that the larval stage is passed in some intermediary host, and VON LINSTOW has lately suggested the millipede (*Julus guttulatus*) in the case of *Ascaris lumbricoides*.

Genus *Heterakis*. Also polymyarian, distinguished from the Ascarides by the presence of a ventral sucker and two often unequal spicules in the male. The male tail has also two series of papillae symmetrically placed, and often two lateral cuticular expansions representing a bursa. Almost all are oviparous. They live in the intestines of vertebrates, particularly of birds. There are several species, found in the fowl, turkey, duck, pigeon, pheasant, bustard, peacock, etc.

The *life history* seems to be simple, at least in the case of *H. taché*, the embryo develops from the ovum in moist media in about seventeen days, and when these ova containing embryos are given to pigeons, adult *Heterakis* are produced in three weeks.

Genus *Oxyuris*. Meromyarian, have three slightly - projecting lips. Oesophagus long with distinct bulb. Males are small and scarce, have a single spicule; two pairs of pre-anal papillae. Females have a long capillary tail, two ovaries, vulva opens in anterior portion of body. Ova are oblong and symmetrical, and often contain an embryo before parturition. Many species inhabit the intestines of man, horse, hare, rabbit, and iguana; and others the rectum of insects, cockroach, water beetle, etc.

The *life history* is simple—the ova, containing developed embryos are taken directly into the alimentary tract, and develop into adult worms.

Genus *Nematoxys*. Meromyarian has very complete arrangement of muscles and forms a transition to the polymyarian type.⁷ The whole body of both sexes is

6. Harmer and Shipley, *The Cambridge Natural History*. Vol. II. P. 138.

7. Harmer and Shipley, *The Cambridge Natural History*. Vol. II. P. 142.

covered with numerous irregularly scattered papillae. There are but few species—found in snakes, amphibia, and eels.

Genus *Oxysoma* has but three species—found in the intestines of opossums, frogs, and turtles.

II. STRONGYLIDAE

Long cylindrical body, seldom filiform or capillary. Mouth surrounded with papillae, probably always six in number; often has an armature of teeth or spines. No distinct oesophageal bulb. The male orifice at the tail end is surrounded by a bell-shaped bursa, with one or two spicules. The female has one or two ovaries: the vulva is sometimes anterior, sometimes posterior to the middle of the body, sometimes near the anus. Ova are already segmented or contain embryos on leaving vagina.

Genera: *Eustrongylus*, *Strongylus*, *Dochmius*, *Sclerostomum*, *Cucullanus*, *Syngamus*, *Pseudalius*, *Ollulanus*, *Oesophagostoma*, and others.

Genus *Eustrongylus*. Cylindrical. Mouth has no lips, but is surrounded by papillae. Male has a filiform spicule; female a single ovary, vulva in anterior part of body.

Only two species known: *E. Gigas*, which inhabits the kidney capsules of carnivorous animals, especially of those which eat fish—dogs, seals, etc., and occasionally man, horse, and deer; and *E. tubifex*, found in aquatic birds—ducks, grebes, divers, etc.

Life history: In case of *E. gigas* the eggs are eaten by fish, the larval stage being passed in the peritoneal cavity of some fishes.

Genus *Strongylus*. Body slender; anterior end sometimes winged. Mouth often indistinctly lipped, has six small papillae. Males have a conspicuous genital bursa, strengthened by variously arranged ridges, which are of specific value. Female posterior end pointed, vulva almost always in posterior half of body.

There are numerous species found in mammals, birds, and reptiles. Some inhabit the intestine; others form aneurisms in the large blood vessels, particularly of horses; others live in the tracheae and lungs of sheep and cattle. They have been found in respiratory tract of the sheep, goat, ox, calf, pig, horse, cat, rabbit, hare, deer, buck, gazelle, ass, dromedary, etc.; in alimentary tract of sheep, goat, chamois, ox, deer, pig, horse, rabbit, etc.; in circulatory system of dog and horse.

Life history: (1) Those of the digestive tract have a rhabditiform embryos provided with an oesophageal bulb, with three chitinous teeth. This embryo lives and grows on the organic matter in mud, and undergo a direct development, (2) Those of the respiratory tract produce larvae with an indistinct oesophageal bulb with no teeth; they do not grow in mud. Their development has not been followed, possibly they have an intermediary host.

Genus *Dochmius*. Anterior end turned towards dorsum. Mouth oval, limited by a chitinous border, followed by a chitinous buccal capsule, the dorsal wall of which is shorter than the ventral, and is supported by a conical rib, the point of which may project into the cavity. At the bottom of the capsule on the ventral wall are two teeth; towards the free edge the ventral wall also bears two other teeth, which are hooked at their extremities. The dorsal free edge is also sometimes similarly toothed. There are several species inhabiting the intestinal canal of man (*D. or Ankylostoma duodenale*), anthropoid apes, dogs, cats, sheep, and goats, wolf, fox, etc.

Life history: According to RAILLIET⁹ and others, the embryos which hatch out, from the already segmented ova a few hours after leaving the intestine, under favourable conditions and after several moults, reach a stage in which they again, on gaining access to the alimentary canal, develop into adult ankylostomes. He mentions that LEICHTENSTERN has asserted that some larvae become transformed into sexually mature rhabditiform adults, which again produce larvae. GILES¹⁰ also reports having traced the life history of the parasite through a sexually mature rhabditiform stage, the larvae of which become adult ankylostomes in the intestine of man.*

Genus *Sclerostomum*. Truncate anterior extremity, straight or slightly curved towards the ventral surface. Mouth circular, open, followed by a chitinous buccal cavity furnished along its edges with numerous teeth, disposed in one or several series. Male has two spicules and a generally tri-lobed caudal bursa. Vulva of female opens in posterior part of the body.

Several species have been found in the intestinal canal of the horse, mule, sheep, goat, deer, roe, antelope, etc.

Life history: RAILLIET¹¹ describes the following in the case of *S. equinum*: the eggs, passed with faeces, develop in water into embryos, which are taken up again probably in drinking water. They probably pass from the intestine into the circulatory system, and after a sojourn there return to the mucous membrane of the caecum, where they remain until a definite stage is reached, whereupon they pass into the intestine and pair. GILES,¹² however, in the case of *S. tetracanthum*, says that rhabditiform adults are produced as in the case of *Dochmius duodenale*.

Genus *Cucullanus*. Exists in the adult form in the intestines of fishes and reptiles. One species (*C. elegans*) lives in fresh water fish, e.g., perch; while the young inhabit the body cavity of the crustacean *Cyclops*.

Genus *Syngamus*. Head end thickened. Mouth large. Chitinous buccal capsule. Males small; two spicules. Females have double ovary; vulva situated in anterior part of body; the male is generally permanently attached to the female, its genital bursa being closely adherent to the vaginal opening.

9. Railliet *Traité de Zoologie Médicale et Agricole*. Paris, 1895. P. 467.

10. Giles *Report on Kala-azar and Beri-beri*. Shillong, 1890.

* The recent researches of one of us (Annett) tend to confirm the truth of these investigations.

11. Railliet, *Traité de Zoologie Médicale et Agricole*. Paris, 1895. P. 459.

12. Giles, *Some observations on the Life History of Sclerostomum tetracanthum*: *Scientific Memoirs by Medical Officers of the Army of India*. Part VII. Calcutta, 1892.

The parasites inhabit the tracheae and bronchi of birds and mammals, fowl, pheasant, turkey, peacock, partridge, magpie, crow, duck, goose, etc.

Life History: The ova escape from the body with fully formed embryos in them, by the decay or rupture of the parent. They hatch in damp earth or water in from one to six weeks, and on being swallowed develop into adults which produce eggs in less than three weeks.

Genus *Ollulanus*. The name is derived from the characteristic appearance of the chitinous buccal capsule, which is urn shaped. The male has two short spicules; the female a single ovary.

One species only is known, *O. tricuspis*, found in the intestine, bronchi, and other parts of the cat. The larvae become encysted in the muscles of the mouse.

Genus *Oesophagostoma*. Small circular mouth has a chitinous ring around which the cuticle is raised into a transparent pad on which are six sharp papillae. The pad is separated from the body by a constriction behind which the integument forms an ovoid swelling well limited posteriorly, at the level of a transverse cleft which occupies the whole breadth of the inferior surface. A few species are known which inhabit the intestine of the ox, horse, chamois, sheep, etc.

Life History: The adults are free in the intestine, the larvae live in small tumours in the mucous membrane.

III. TRICHOTRACHELIDAE

This family is characterised by the anterior end of the body being long and whiplike, the posterior somewhat swollen. The mouth has no papillae; there is no oesophageal bulb. Males may have no spicule, or more often a single spicule surrounded by a sheath. The females have a single ovary; the vulva is situated at the beginning of the thicker portion. Some are ovoviviparous, others oviparous. Their eggs have two characteristic poles.

Genera: *Trichocephalus*, *Trichosoma*, *Trichina*, and others.

Genus: *Trichocephalus*. The anterior and posterior parts well marked. The ventral surface shews anterior by a broad longitudinal band formed by a number of punctiform projections. The male tail is twisted spirally, with its concavity corresponding to the dorsal surface. The female has a single ovary.

Several species are known, inhabiting the intestine of man (*T. dispar*), monkeys, lemurs, swine, hog, peccary, dog, cat, sheep, deer, ox, etc.

The *life history* is simple; there is no intermediary host.

Genus: *Trichosoma*. The posterior part containing the intestine and generative organs, is but very little swollen. The posterior end of the male has no papillae, but bears a rudiment of a bursa.

Parasites of birds and mammals. In mammals, different species live in the bladder of the fox and wolf, and of the cat, and in the trachea of the fox and martin. In some species two, three, or four males live within the uterus of the female.

Genus *Trichina*. Small capillary worm, slightly swollen posteriorly. Male has two conical appendages posteriorly forming a sort of copulatory bursa. There is no spicule. Female is viviparous. Vulva situated in anterior fifth of the body.

A single species *T. spiralis* only known.

Life history. The adults, male and female, live in the intestine of man and other mammals. The female produces very numerous eggs which give rise to embryos in the body of the uterus. These embryos bore through the intestinal wall of their hosts, and make their way all over the body, coming to rest most usually in the muscles. Here they generally pierce the sarcolemma and become encysted inside the muscle fibre. The larvae may here remain dormant for many years, and undergo fatty or calcareous degeneration. When trichinised meat is eaten, unless thoroughly cooked, the cysts are dissolved and larvae set free, and become sexually mature in three or four days; again producing ova and embryos which bore through the intestinal wall.

IV. FILARIIDAE

Long filiform worms; mouth with two lips or without lips—often have papillae, and sometimes a buccal capsule. Males have a tail generally incurved, have one or two unequal spicules, four pairs of pre-anal papillae, and sometimes an unpaired one as well. Females have double ovary. Vulva is situated towards the anterior part of the body. Many are ovoviviparous.

Genera: *Filaria*, *Ichthyonema*, *Hystrichus*, *Spiroptera*, *Disparagus*, and others.

Genus *Filaria*. See next chapter.

Genus *Ichthyonema* is confined to fishes. Male is very minute, and the female partly degenerate. No anus, no external generative organs. Uterus fills the entire body cavity.

Genus *Hystrichis*. The anterior part of the body is armed with spines. Male has a bell-shaped bursa, and very long spicule. Vulva is near the anus.

The parasite lives between the walls of the oesophagus and gizzard of some birds—palmipeds (duck, swan).

Genus *Spiroptera*. These can only be distinguished from the *Filariæ* by two features—the body is generally shorter and thicker, and the vulva is ordinarily nearer the mouth. Their specific name is taken from the tail of the males, which is rolled into a spiral and furnished with lateral membranous expansions.

Several species are described generally met with in tumours of the oesophagus, stomach and intestines of horses, asses, mules, pigs, dog, wolf, etc. *S. reticulata* has been found in the cervical ligament, in periarterial tissue, between muscles and tendons, and in other positions in the horse.

Life history is unknown—an insect is supposed to act as an intermediary host.

Genus *Dispharagus*. These have the oesophagus divided into an anterior straight tubular portion, and a long thick muscular posterior portion with a bulb. Male tail extremity is more or less coiled, and has lateral expansions : four pre-anal papillae on each side, two unequal spicules. Female has a simple ovary, and is oviparous. The several species occur in the oesophagus and stomach of some birds.

V. MERMITHIDAE

Mouth has six papillae. There is no anus. Males have two spicules and three rows of numerous papillae. Body of female reduced to a simple sac, crowded with ova.

Genera : *Mermis*, *Bradydema*, *Atractonema*, *Allantonema*, *Sphaerularia*, etc.

These are parasitic in some stage on insects, *e.g.*, the sexually mature forms of genus *Mermis* live in damp earth, while the larval stage find their way into grasshoppers, caterpillars, etc. The adult stage of *Bradydema* live in the body of small beetles, then reach the intestine, and eventually earth, where the females die, and the males, having developed spermatozoa in the larvae stage, now develop ova (protandrous hermaphroditism). The *Allantonema* have a somewhat similar history, as have also, *Atractonema* and *Sphaerularia*. The two last have the peculiar feature that at the time of sexual maturity a swelling—a prolapsus of the uterus and vagina—develops posteriorly and grows until it far exceeds the size of the worm. *Sphaerularia* are parasitic in the body cavity of many bees (*Bombyx*).

VI. ANGUILLULIDAE

These are for the most part free living and small. Oesophagus has two bulbs, the posterior without teeth. Buccal cavity contains a small spine. Males have sometimes a bursa with no papillae ; two equal spiculae. Females have a double ovary, and vulva in posterior half of body ; often ovoviviparous, but the number of embryos is small.

Genera : *Diplogaster*, *Mononchus*, *Rhabditis*, *Tylenchus*, *Anguillula*, *Heterodera*, etc.

Many species of this family live in humus or decaying matter, others are parasitic in plants ; some live in organic matter, and some few are parasitic in animals.

Tylenchus, *Aphelenchus*, and *Heterodera* infect plants and give rise to 'sickness' among clover, ryé, oats, onions, beet, etc.

Genus *Rhabditis*. Oesophagus has two bulbs, posterior, and sometimes with teeth. Buccal cavity no teeth nor spines. Males may have a caudal bursa ; often has six to ten papillae on bursa or in middle line, two short spicules with an accessory piece. Some species are hermaphrodite.

Some species live in moist earth, others are described as causing a disease resembling typhoid, and larvae of species have been formed in the papules of some skin eruptions in man and dogs.

Genus *Anguillula*. Oesophagus has two bulbs, posterior has no teeth. Male is provided with a bursa with no papillae. The uterus is asymmetrical.

Numerous species are parasitic on plants, wheat, etc. *Anguillula aceti* is found in vinegar and paste. Others present two mature generations which succeed each other, (1) a free form, dioecious, resembling Rhabditis, and (2) a hermaphroditic form which is parasitic.

OERLEY places these in a new family, *Angiostomides* with three genera—*Angiostoma*, *Strongyloides* and *Allantonema*.

Anguillula intestinale (*Strongyloides intestinale*, *Anguillula stercoralis*) is parasitic in the intestine of man, giving rise to some forms of diarrhoea and dysentery (Cochin China), and produces ova which give rise to rhabditiform larvae, which are passed with the faeces. In the soil these become sexually mature, pair and produce larvae, which eventually reach the digestive tube to become *Anguillulae intestinale*.

VII. ENOPHIDAE

These are free living, small, usually marine; devoid of a second oesophageal bulb. Eyes and mouth armature often present. Fine hairs and bristles surround the mouth.

Genera: *Enoplus*, *Dorylaimus*, *Encelidium*, etc. Some species are parasitic on plants, others on the sea urchin, and other animals.

II. THE *FILARIAE*

The genus *Filaria* is a very large one. It appears to be confined to vertebrates, usually living in the tissues of the body and not in the intestines. The worms are remarkable for their long slender bodies, which are almost of the same breadth throughout the whole length. The anterior extremity is rounded, and often has no lips. The males, which are markedly smaller than the females, have an incurved or spiral tail sometimes furnished with lateral expansions; more often they possess four pre-anal, and a variable number of post-anal papillae; the spicules vary considerably in size and appearance. In the females the vulva opens more or less near the mouth.

FILARIA WHICH ARE PARASITES OF MAN

Filaria bancroftii

Filaria diurna

Filaria perstans

Filaria ozzardi

Filaria magalbesi

Filaria demarquaii

Filaria loa

} These will be described in a subsequent chapter.

Filaria medinensis

Guinea worm: The adult female is a white or yellowish worm, averaging about sixty centimetres long, though specimens reaching four metres in length have been described. Its breadth, which is uniform, is from 0·5 to 1·7 mm. The anterior extremity, which tapers slightly, is truncated, and presents a rugous cuticular thickening in the centre of which is the triangular buccal orifice. The thickening bears two large papillae, one dorsal and one ventral, and six small papillae. The body shows faint transverse striation. The cuticle is thick. The musculature is polymyarian. The tail incurved towards the ventral surface in the matured females, terminates in a sharply bent hook about 1 mm. in length. The alimentary canal consists of a fine tube running from the mouth to near the tail, but not opening externally in the gravid female, though an anal orifice exists in the young parasite. In the mature worm the uterus crowded with embryos fills the whole body cavity—vulval opening and vagina being obliterated. The embryos, usually lying curved on themselves *in utero*, measure 15 to 25 μ long by 0·50–0·70 μ wide. They are slightly flattened, transversely striated, and provided with a finely tapering tail which measures about two-fifths of their whole length. They have a rudiment of an

alimentary canal, and bear two small lateral sack-like structures at the base of the tail. They swim actively and may live for days in muddy water and damp soil. They are said by some authors to escape only by rupture of the adult worm, but according to MANSON¹ they are emitted by a prolapsus of the uterus through the mouth. The mature worm drills a hole in the derma. Over this the epidermis forms a bulla, which ruptures in a few days, disclosing a small superficial ulcer with a hole at its centre, under which lies the head of the worm. On the application of water to the ulcer, a minute quantity of whitish fluid is extruded, seen on microscopical examination to be swarming with embryos; or a little tube, the prolapsed uterus itself, is sometimes seen protruding. In about a fortnight the whole uterine contents are emptied. It is usually asserted that the female alone is known, and that it is uncertain whether it is hermaphrodite or whether both sexes are present in the *Cyclops*. CHARLES² has described a specimen found in the mesentery of a human subject from an orifice in the middle of the body of which he drew out a much smaller specimen, which may have been the male.

Life history. The young embryos in water attack a fresh-water *Cyclops* and penetrate through the interarticular membrane between the abdominal plates into the body cavity. Here the intestine of the parasite further develops, and on the eleventh day they moult and exhibit a very changed appearance, being shorter (0.5 mm.) and non-striated. In four weeks they measure 1 mm. in length. They are thought to reach man again through the medium of drinking water containing infected *Cyclops*: the parasite being able to pierce the tissues to reach its usual site in the legs. CHAPOTIN and others claim that the embryos can enter the body through the skin. PLEHN³ reports to have fed two monkeys on bananas covered with embryos, and that one of them subsequently developed a painful tumour of the thigh and died after eight and a half months. The tumour contained a worm in all respects identical with *F. medinensis*, though only 4.0 cm. long.

***Filaria lentis.* Diesing**

Syn. *F. oculi humani*, VON NORDMANN. Under this title are included nematodes, found on several occasions in the eye of man. Those described have varied considerably in length, 1.72 to 12.6 mm. RAILLIET⁴ considers that they represent worms of different species which have gained access to the wrong host, or such as have been arrested in their development. Specimens have been described by VON NORDMANN, GESCHEIDT, and SCHÖLER.

***Filaria inermis.* Grassi**

Syn. *F. palpebralis*, PACE, nec WILSON; *F. peritonei hominus*, BATES; *F. conjunctivae*, ADDARIO. The female only is known. It measures about 160 mm. long

1. Manson, *Tropical Diseases*. 1900 p. 554.

2. Charles, a Contribution on the Life History of the male *Filaria Medinensis*, founded on the examination of specimens removed from the abdominal cavity of man. *Scientific Memoirs, by Medical Officers of the Army of India*. Part vii. Calcutta, 1898.

3. Plehn, *Die Kameru-Küste*, etc. Berlin, 1898. p. 295

4. Railliet, *Traité de Zoologie Médicale et Agricole*. Paris, 1895. p. 529.

by 0.475 mm. broad. It is of whitish or brownish colour, somewhat flattened, threadlike, and tapers slightly towards both extremities, but more especially posteriorly. The extremity of the tail is incurved. Cuticle is transversely and longitudinally striated, except at the cephalic end; musculature polymyarian. The mouth is very small, unarmed, and terminal; oesophagus is short (620 μ), widens somewhat at hinder end. The anal aperture is 300 μ from the tip of the tail, the vulva 50 to 104 μ from the mouth. The eggs hatch out in the uterus; the free embryos measure 350 μ by 5.5 μ , and taper slightly in front, sharply pointed posteriorly. A peculiar formation, probably of glandular nature, occurs at the point of the tail where the cuticle is thin: on each side of the thin portion is a break, with a corresponding canal which resembles the duct of a gland. In some examples there is a third one between the other two, but its outer opening was not made out.

Life history unknown. The adults have been found in man, the horse, and donkey. In man they have been found three times in the eye: once encysted in the gastro-splenic omentum, and once encysted in the ocular conjunctiva. *Filaria lentis* (DIESING) may be a young form of this worm.

***Filaria volvulus.* Leuchart**

The description of the male and female of this worm is given by PROUT.¹ The female is 40.4 cm. long and the body 0.34 mm. wide, which gradually tapers to the head end which is 0.04 mm. across, and to the tail, where the diameter is 0.0084 mm. The cuticle is striated, the tail end slightly curved. Anal orifice was not made out. Alimentary canal simple. The double uterus was observed to commence at a distance of 4.35 cm. from the tail end in a sacculated extremity. The ova, containing coiled up embryos, measure 0.032 by 0.034 mm. Embryos are 0.18 to 0.2 mm. long and 0.001 broad.

The male is smaller and thinner than the female, being on an average 3.14 cm. long, and 0.44 mm. broad. The worm is white and flattened somewhat, has a striated cuticle, and is uniformly tapered towards each end. The diameter of the head is 0.044 mm., and of the tail posterior to the anal orifice 0.028 mm. The head is rounded; tail markedly incurved. The alimentary canal is simple. The anal orifice is at a distance of 0.049 mm. from the caudal extremity. The extreme end of the tail on the concave side is flattened, and here four papillae were made out. The anal orifice itself seems to have two lateral, one post- and one pre-anal papillae on each side. Two unequal spicules—one protruding was slightly clubbed at the extremity, and trumpet shaped at its inner end; the other commencing just within the orifice was much longer than the first, and of much the same shape, but narrower at the point. A narrow, central canal was observed in the former which has a minute opening at its free end.

1. Prout, *British Medical Journal*, January 26, 1901, p. 209.

Life history unknown. The adults occur in pairs in subcutaneous tumours, the purulent contents of which swarm with embryos. These are 0.25 mm. long, by 0.005 mm. broad, have a rounded head, sometimes a double tip. Their tails are sharp and granular. They have no sheath. In stained specimens a V-shaped spot can be made out at the junction of the anterior fifth with the posterior four-fifths.

***Filaria labialis.* Pane**

The female only is known. It is about 40 mm. long, slender, tapering at each end, but slightly swollen at the extreme posterior end. The mouth is surrounded by four papillae. The anus is at 0.5 mm. distance from the posterior end, while the vulva opens at 2.5 mm. more anteriorly. The uterus is double.

Life history unknown. A single specimen only has been seen in a small pustule on the inner side of the upper lip.

***Filaria hominis oris.* Leidy**

Length 140 cm., breadth 0.16 mm. A filiform, opaque white worm, with a simple round mouth, and blunt tail furnished with a short hook 50 μ in length and 12 μ across at the base.

Life history unknown. A single specimen has been found in the mouth of a child. It is thought by LEIDY and LEUCHART to be an immature *F. medinensis*.

***Filaria lymphatica.* Treutler**

Syn. *Haemularia lymphatica*, TREUTLER; *F. hominus bronchialis*, RUDOLPHI. Length, 26 mm.; brownish in colour, speckled with white, almost transparent posteriorly. Body filiform, a little compressed laterally.

Life history unknown. Found in hypertrophied lymphatic glands. DIESING and WEINLAND regard it as identical with *Strongylus longevaginatus (paradoxus)*. RAILLIET suggests it as a male *F. inermis*.

***Filaria restiformis.* Leidy**

A single specimen only found, passed *per urethram*. Length, 65 cm.; breadth, 1.5 mm. Long, uniformly cylindrical body. Cuticle smooth, no transverse striation; head end tapering somewhat, rounded, no appendages. Caudal end incurved, no appendages. No apparent anal or genital aperture. RAILLIET considers this a pseudo-parasite.

SOME FILARIAE WHICH ARE PARASITES OF ANIMALS

***Filaria equina.* Abildgaard**

Syn. *Gordius equinus*, ABILDGAARD; *F. equi*, GMELIN; *F. papillosa*, RUDOLPHI; *F. equina*, BLANCHARD.

A whitish filiform worm tapering towards the extremities especially posteriorly. Cuticle finely striated transversely. Mouth small, round, provided with a chitinous ring, the edge of which has laterally two crescentic lips, and at a point corresponding

to the dorsal and ventral median lines, a simple or indented papilla ; behind the ring are four submedian papilliform chitinous spicules.

The male is 6-8 cm. long, has a spiral tail, with four pre- and four post-anal papillae, and two unequal spicules.

The female is 9.12 cm. long, has a slightly spiral tale terminating in a rounded button, in front of which are two lateral conical protuberances. The worm is viviparous ; embryos measure 280μ by 7μ wide.

The embryos have been observed in the blood of animals found afterwards to contain the adult forms. They are one-seventh mm. in length, and one to three occurred in each drop of blood. They resemble the embryos of *Filaria sanguinis hominis*, but are much smaller.

Life history is unknown, but it is surmised that development takes place in the body of an insect host. The adults have been found in the peritoneal cavity, tunica vaginalis, fallopian tube, pleural cavity, between the dura and pia-mater, in the aqueous humour, in the intestine, and in the liver of horses, donkeys, and mules.

***Filaria labiato-papillosa.* Alessandrini**

Syn. *F. cervina*, DUJARDIN ; *F. terebra*, DIESING. This species resembles the preceding in its appearance and dimensions. Mouth is oblong dorsoventrally, surrounded with a chitinous ring, the edge of which supports four curved projections. On the median, dorsal, and central line, the chitinous ring forms a papilliform spine, markedly double in the female. Behind the mouth are four small sub-median depressions, from each of which a tactile papilla arises. The male is 6-8 cm. long ; tail is closely spiral ; has three pre-anal, one ad-anal, and five post-anal papillae on each side, and behind these a conical projection. The female is 6-12 cm. long ; has a spiral tail, terminating in a number of small blunt points which arise from two lateral conical protuberances. The worm is viviparous ; embryos 140 to 230μ long.

Life history is unknown. The adults have been found in the peritoneal cavity of cattle and deer.

***Filaria haemorrhagica.* Railliet**

Syn. *F. multipapillosa*, CONDAMINE and DROUILLY ; *F. multipapilla*, MOLIN. White cylindrical body, slightly tapering at the extremities, more so behind than in front. Anterior extremity has a retractile cone. The integument is transversely striated. The striations near the anterior extremity become broken, and form elliptical or circular depressions, and a large number of papilliform projections. The mouth is simple, circular.

The male is about 28 mm. long, 0.26 broad ; posterior extremity is rounded, there are two unequal spicules, one $680-750\mu$ long, the other $130-140\mu$.

The female has a length of 42-70 mm., and breadth 0.42-0.44 mm. ; caudal end rounded : the vulva is near the mouth. The ripe eggs are from $52-58\mu$ long, 24 to 33μ wide, and contain an embryo.

The free embryos measure 220-230 μ long by 9-11 μ wide.

The *life history* is unknown. The male and female live together in the connective tissues of the horse and donkey giving rise to hemispherical protuberances about the size of a nut, beneath the skin. These quickly burst and allow blood to escape, after which they subside and appear again in twenty-four to forty-eight hours in other places. Tracts of the worm can be seen in many tissues post-mortem. It is surmised that the embryos are taken up by some insect or crustacean.

***Filaria immitis*. Leidy**

Syn. *F. canis cordis*, LEIDY; *F. papillosa*, *haematica canis domestici*, GRUBY and DELAFOND.

Body white, filiform, a little tapering at each extremity especially posteriorly. Anterior extremity rounded. Mouth terminal, small, simple, surrounded by six small indistinct papillae. Anus near the end of tail.

The male 12 to 18 cm. long, 0.7 to 0.9 mm. broad, with spirally wormed tail bearing two small lateral ridges supported by papillae, four of which are larger than the others—there are three pre- and one post-anal papillae, MANSON¹ however describes the arrangement of papillae differently. Two unequal spicules.

The female is 25-30 cm. long, 1 to 1.3 mm. broad. The tail is short, blunt and curved; vulva is at a distance of about 7 mm. from the mouth. The ova hatch within the uterus: the free embryos measure 285 to 295 μ by 5 μ ; their anterior extremities are slightly tapered and end bluntly, the posterior tapers gradually to a fine point. The embryos occur in large numbers in the blood of the infected animal. MANSON observed a certain degree of periodicity, the embryos being most numerous in the peripheral blood at night, not disappearing entirely however during the day.

Life history. The adult parasites are found chiefly in the right ventricle of the heart of the dog, fox, and wolf.

The development of the embryos has been the subject of many investigations. BANCROFT affirmed that he found the embryos in the intestine of *Trichodectes* which had sucked the blood of infected dogs, and supposed these insects to play the part of intermediary host. SONSINO confirmed this, but recognized later that *Trichodectes canis* does not suck blood and that *Haematopinus pilifer* was meant. GRASSI and SONSINO found larvae of Nematodes in the intestine and body cavity of dog fleas, and concluded they were dealing with the embryos of either *Spiroptera sanguinolenta* or of *Filaria immitis*. Subsequently it was found that Spiroptera do not give rise to haematozoal embryos, and it was inferred that dog fleas were the intermediary hosts. Later GRASSI conclusively proved that neither *Pulex serraticeps*, *Haematopinus*, nor ticks (*Rhipicephalus siccus*, КОСН) served as the hosts for *F. immitis*. In the previous

1. Manson, *Medical Times and Gazette*, 1877. Vol. II. page 513. etc.

investigations, he claimed that SONSINO was led astray by the coincidence that *F. recondita* was present in the dogs he examined, the embryos of which were mistaken for those of *F. immitis*. GRASSI then thought the intermediary host to be a crustacean or mollusc.

However in 1900 he¹ describes the development of these embryos inside the mosquito. 'The embryos sucked up by *Anopheles* migrate into the malpighian tubes, where they continue their development behaving more or less like the other blood filariae already known. The larvae, arrived at maximum development, abandon the tubes and enter the general body cavity leaving behind the old cuticle: there they progress towards the head and collect there rapidly in the prolongation of the general body cavity within the labium (called also the inferior labium), occasionally in the palpaе.' In their experiments these authors seem to have allowed a period of thirteen or fourteen days for the complete development of the embryos in *Anopheles*. They do not appear, however, as far as we have been able to ascertain to have carried out the infection of healthy dogs by the bites of infected *Anopheles*. One experiment is described, undertaken on July 19, 1900, in which a healthy dog was injected subcutaneously with larvae, collected in a drop of normal saline solution, from the labium of two infected *Anopheles*. At the post-mortem on August 4th (a period of sixteen days) there was found 'in the subcutaneous tissue near the genitals, a very small female filaria which must be judged *Filaria immitis*, still immature. We were able to preserve only its anterior half sufficiently for diagnosis.' This does not seem to us very satisfactory; details of the appearance and anatomy of this anterior half of an immature *Filaria immitis* not being given.

***Filaria recondita*. Grassi²**

The female only is known. This is about 3 cm. long, 0.178 mm. broad. The transparent body tapers towards both ends, more especially posteriorly. The integument is nonstriated. The anterior extremity is obtuse, bears at least four very small papillae close to the buccal orifice. Posterior extremity is also blunt, and has three papillae, one terminal and two lateral, and also several small papilliform projections. The mouth is followed by a very short cylindrical oesophagus, somewhat less than 2.5 mm. long. The anus is at a distance of 228 μ from the tip of the tail. The uterus is double, the vulva at a distance of 840 μ behind the mouth.

Life history. Up to the present only a single female specimen (which was immature, containing neither embryos nor eggs) has been met with. It was found coiled up but not encysted in the adipose tissue near the hilum of the dog's kidney. The embryos have been studied by GRUBY and DELAFOND, LEWIS, MANSON, GRASSI, SONSINO, and others, in France, China, India, and Italy.

1. Grassi and Noé, *British Med. Journal*, 1900. Nov. 3, p. 1306.

2. In a footnote in his article on 'Filariasis' in the *Encyclopaedia Medica*, Vol. III, Nuttall says: 'Sonsino (personal communication, December, 1899) considers it doubtful that this is a "good species," the determination having been made upon a single female specimen.'

GRASSI and CALANDRUCCIO¹ traced out the development of the embryos in *Pulex serraticeps* (of the dog and cat), *Pulex irritans* (of man and dog), and *Rhipicephalus* *siculus*, KOCH (a dog-tick). They describe the following stages:—

First Stage. Embryo found in the blood of dogs, and in the intestine and body cavity of fleas. Length 280μ , breadth 5μ . Body slightly thinned in front, but ending bluntly: behind it tapers and ends in an almost hair-fine point. It is smaller than the embryo of *F. immitis*, and possesses the characteristic that they fix their oral end to the coverglass. At the front end can be made out a fine canal, representing the oesophagus. In those which have reached the body cavity there can be made out a certain trace of the intestinal tract and of the anus. The embryo executes snake-like movements.

Second Stage. Found in the fat cells, seldom free in the body cavity. The larvae of the previous stage first shorten without thickening, then thicken and finally lengthen. The cells of the larvae are larger, and the organs more distinct. The body is cylindrical, and in front has a finger-like papilla 5.6μ long, covered with cuticle somewhat thickened at the free end, and appearing as though filled with a clear liquid. Long pointed tail. Parts of the alimentary tract are becoming differentiated. The genital apparatus is just appearing. The worm has no movement.

Third Stage. A moulting of the cuticle takes place either in the cell or when free in the body cavity. Length reaches 1.5 mm . The front end is blunt, the papilla of the previous stage disappears. Hind end has three papilla, one terminal dorsal, two other almost terminal, and ventral. The fine point of the tail has disappeared. Further development of the alimentary organs—mouth opening has four papillae. The worm shows active eel-like movements.

Fourth Stage. Only once seen. The larva was encysted, and was considerably larger and thicker. Genital apparatus developed. The tail, besides the papillae, bears a little process (as in adult).

Stages three and four are similar to the adult, and much further development cannot take place.

Attempts, however, to infect dogs with infected fleas failed.

***Filaria irritans*. Rivolta**

Syn. *Dermofilaria irritans*. This name is given to a nematode larva, which measures about 3 mm . in length; its head is slightly marked off by a neck from the body; the tail tapers and terminated in a blunt notched point. The mouth is round, and appears to be provided with lips. At a little distance from the head end an opening is seen. The anus occurs at the point where the body tapers into the tail. The integument bears fine transverse striations.

The *life history* is unknown. These larvae are found in the 'summer sores' or 'granular dermatitis' of horses and donkeys.

1. Grassi and Calandruccio, *Centralblatt für Bakteriologie*, 1890, vii, 18-26

***Filaria evansi.* Lewis**

A description of this species¹ of which the male and female are known, is not procurable. The worms were found in the lung and mesentery of a camel at Madras, the pulmonary arteries being obstructed by masses of tangled worms—the blood containing number embryos similar to those of *F. bancrofti*.

***Filaria lachrymalis.* Guret**

Syn. F. bovis, BAILLET. *F. palpebrarum*, RAILLET. A whitish cylindrical worm, slightly tapering at each end. Cuticle transversely striated. Mouth small, simple, followed by a cylindrical buccal cavity. Anus almost terminal. Male 10-14 mm. long, tail bowed, has two very unequal spicules. Female 15-24 mm. long, has a straight conical tail. Vulva about 1 mm. from anterior end. Ova ellipsoid, hatch inside the uterus. Embryos 210-230 μ long.

Life history unknown. The adults live in the lachrymal-duct of cattle.

***Filaria palpebralis.* Wilson**

Has a white cylindrical body, slightly tapering at each end. Cuticle has fine transverse striations. Small, simple mouth. Anus almost terminal. Male 8-12 mm. long, tail curved; bears three pairs of post-anal of papillae and two unequal spicules. The female is 14-22 mm. long, and has a straight conical tail; the vulva is at 0.60-0.70 mm. from the anterior end. Ova are ellipsoid hatch under the uterus. Embryos have a length of 120-170 μ .

Life history unknown. The adults have been found in the excretory ducts of the lachrymal glands and under the eyelids of the horse.

***Filaria osleri.* Cobbold**

Syn. Strongylus bronchialis canis, OSLER.

Body filiform; mouth surrounded by two or three lips behind which are three unequal papillae; pharynx swollen. The male is 5 mm. long and has a rounded posterior end; and two unequal curved spicules. The female is 9-15 mm. long, tapers at each end; anus almost terminal; vulva immediately in front of anus; the worm is ovoviviparous.

Life history unknown. The adults were found by OSLER to be the cause of an epizootic bronchopneumonia in dogs at Montreal. RABE and RUMBERG had previously observed the worm in small nodules in the mucous membrane of the respiratory passages, each nodule containing several male and female worms.

1. Lewis. Remarks on a Nematoid Haematozoon discovered by Dr. Griffith Evans in a Camel. *Proceedings of the Asiatic Society of Bengal*, 1882, p. 63.

Filaria clava. Wedl

The female only is known—length 16-18 mm., breadth 0.33 mm. Body filiform and of uniform thickness throughout almost the whole length. Head end conical; posterior end rounded and bulbous. Mouth simple, small. Anus in a groove at the bulbous end. Vulva at 1.25 mm. from the anterior end. Ova $36\ \mu$ by $24\ \mu$ contain a coiled-up embryo. Embryo $84\ \mu$ long, $6\ \mu$ wide, thin rounded anterior end, pointed posterior end. Found in the peritracheal connective tissue of the domestic pigeon.

Filaria mazzanti. Railliet

The female which alone is known is 25 mm. long, 0.25 wide; has a rounded anterior end, conical posterior end. Mouth simple, round. Anus terminal. Vulva triangular, $213\ \mu$ from anterior end. Viviparous. Found under the skin of the neck of a pigeon, whose blood contain embryos some $185\ \mu$ long with slightly pointed tail, the others $142\ \mu$ long with blunt tails.

Filaria uncinata. Rudolphi

Syn. *Spiroptera uncinata*, RUDOLPHI; *Dispharage à queue crochue*, RAILLIET; *F. uncinata*, RUDOLPHI. Mouth has two lips with six papillae. The four sinuous cutaneous bands (characteristic of the *Dispharagi* RAILLIET¹) reach to within 2 mm. of the anterior end. On each side of the body a double longitudinal series of small spines extends almost to the caudal extremity; in front, the spine ridges reach the dorsal surface and approach the mouth between the cutaneous bands.

The male is 9-10 mm. long; the tail shows straight lateral alae with vesicular edges. Four post-anal papillae; the pre-anal five or six side by side, or seven or eight; the principal spicule is long, incurved and dilated at its free extremity; the other is thick and short.

The female is 15 to 18 mm. long; vulva is at about 1 mm. from the caudal extremity, which is curved.

Life history. This has been worked out by HAMANN² in *Daphnia pulex* (RICH). The adults occur in the oesophagus and ventriculus of geese and ducks in tubercles of different sizes which contain worms up to about 10 mm. in length, coiled together. The disease attacks the younger animals of late generations; those of the first brood are unaffected, explained by the course of the development of *Daphnia*. This crustacean multiplies the whole year round, but mostly in the hot summer months, especially of July and August. The mature worms give rise to embryos which wander out of the tumour and may, either, come out by the oesophagus and mouth, or, more usually passed through the intestine, and escape

1. Railliet, *Zool Medic et Agric.* Paris, 1895, p. 542.

2. Hamann, *Central. f. Bakt. u. Paras.*, 1893, xiv, p. 555.

per rectum. They are then taken up by *Daphnia*; bore through the intestine and lie in the body cavity, reaching 1.7-2 mm. in length. They show a typical mouth with six papillae and a 'vestibulum'; only the generative organs are lacking. They are then swallowed by ducks and bore into the wall of the stomach and oesophagus. STOSSICH does not include this worm in his list of *Filariae*.

***Filaria picae mediae*. Manson¹**

The male and female were found coiled up in a small white tubercle in the pocket of the semi-lunar valve; the worms were encysted, and a minute opening in the covering of the cyst may be present.

The male is about 18.5 mm. long; diameter at the neck about 0.06 mm.; greatest diameter about 0.18 mm., of alimentary canal about 0.06 mm., and of spermatic tube 0.08 mm. The tail is strongly incurved. Two spicules. One or two minute caudal papillae. The tail is blunted and slightly lobed and tapers down from the body. The mouth is simple; oesophagus straight about 0.5 mm. long, terminates in the alimentary canal by a gradual dilatation. Alimentary canal is straight, and filled with a dark granular material. The integument is covered with minute bosses or tubercles, largest about the middle of the animal, less marked towards the head and tail ends.

The female averages about 37 mm. in length; greatest diameter of unimpregnated specimen about 0.2 mm.; impregnated about 0.3 mm. The anus is at about 0.12 mm. from the caudal extremity. The vagina is infundibuliform, and opens at 0.25 mm. from the mouth. Mouth, oesophagus and alimentary are similar to those of the male. Expressed embryos measure 0.12 mm. long by 0.004 mm. broad. They have no sheath; tails are truncated.

In the blood of this bird, MANSON found embryos apparently of two kinds, one about 0.1 mm. long; the other about 0.22 mm.; intermediate sizes were also present. The smaller were languid, the larger very active in the movements. A jerking pouting oral movement was seen in both. Tails sharp and pointed.

***Filaria corvi torquatis*. Manson**

The adults occur in the right ventricle, pulmonary artery and its branches.

The male is about 0.3 mm. long; greatest diameter about 0.16 mm.; diameter of neck 0.06 mm. Length of oesophagus 0.6 mm.; diameter 0.04 mm. The body is smooth and very transparent. Mouth simple; spicules double. No papillae. Tail tapers to a blunt extremity. Anus close to end of tail.

Female 18-20 mm. long; diameter 0.27 mm. Vagina opens 0.25 mm. from mouth.

The blood of the bird contained two dissimilar embryos; the larger 0.21 to 0.25 mm. long, and 0.008 mm. broad; the smaller 0.13 mm. long, 0.004 mm. broad.

1. Manson, *Journal of the Quekett Club*, vol. xi, 1880, p. 130.

The former showed active, lashing, free, vigorous movement; the latter languid, slow wriggling. Oral movements were distinct; there were four papillae round the mouth. The tail of the larger tapered and was pointed; of the smaller, tapered slightly, was truncated, and a thin skin extended like a loose bag or hood from the head end.

MANSON also gives meagre descriptions of haematozoal embryos in *Gracupica nigricollis* and *Goura coronata* (Malay Archipelago).

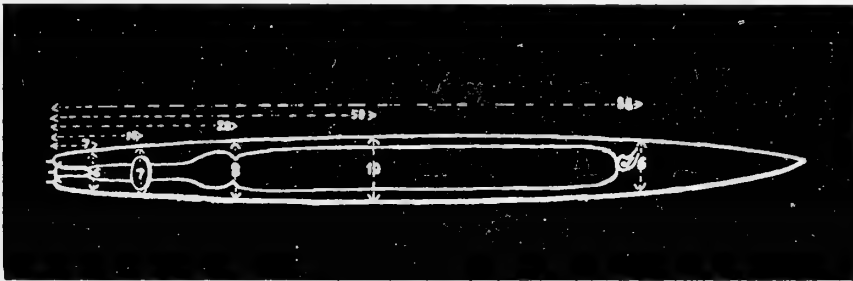
STOSSICH¹ describes in his monograph 212 species of *Filariae*—we have arranged a complete list of these from his work, giving their hosts, and sites, and also the literature referring to each. This list will be found in the Bibliography.

1. Stossich, *Filarie e Spiroptere*, Trieste, 1897.

III. AVIAN FILARIAE.—NEW SPECIES

This description of new blood filariae discovered in West African birds of different species includes the account of eight new species, of which the adult forms generally both male and female were found, as well as the blood embryos, and also of three species in which blood embryos alone were met with.

With a view to obtaining some uniformity in the descriptions and measurements of Nematode worms in general, COBB¹ has devised an ingenious formula, for the account of which we are indebted to SHIPLEY², in which measurements of different parts appear as percentages of the whole length of the body. The following diagram explains the nature of the formula, which, however, should be used with caution since it rests on the assumption, as SHIPLEY² points out, that the proportions of the various parts of the body are constant in different individuals, and it is by no means certain that this is the case.



In the diagram, 6, 7, 8, 10, and 6 are the transverse measurements, while 7, 14, 28, 50, and 88 are the corresponding longitudinal measurements. The formula in this case is

$$\frac{7 \quad 14 \quad 28 \quad 50 \quad 88}{6 \quad 7 \quad 8 \quad 10 \quad 6}.$$

The unit of measurement is the one-hundredth part of the length of the worm, so that the measurements are therefore percentages of the length. The measurements are taken with the animal viewed in profile; the first is taken at the base of the oesophagus, the second at the nerve ring, the third at the cardiac constriction, the fourth at the vulva in the female and at the middle in the male, the fifth at the anus.

1. Cobb, *Macleay Memorial Volume*, Sidney, 1893, p. 252; and *Proc. Linnean Society*, N.S.W. Second series, Vol. V, 1890, p. 449.
2. Shipley, Harmer and Shipley, *Cambridge Natural History*, Vol. II, *Worms*, etc., p. 138.

This plan will be followed as nearly as possible in the following descriptions :—

***Filaria cypseli*. Nov. Sp.**

Definitive host—*Cypselus affinis*. The West African swift. The infected birds were found to have built their nests among the rafters supporting the verandah of the telegraph station of the African Direct Telegraph Company at Bonny, Southern Nigeria ; and in the neighbouring palm trees.

Site. The adult filariae were found in the subcutaneous tissues of the head and neck. In one bird six worms occurred, four of which were mature females, one an immature female, and the sixth a mature male ; in another two females and one male. Some came off with the skin on stripping the scalp ; two were found in the neck, one of them extending as far down as the middle of the back. They were not coiled up but lay more or less straight among the subcutaneous tissues. They were observed to move in the tissue with a slow sinuous motion backwards and forwards, and could be kept alive in normal salt solution for about ten hours.

The adult worms are very long and thin, white in colour. The cuticle shows faint transverse striations. The female has an average length of 25.3 mm.—the length varying in our specimens from 24.0 to 26.7 mm. (the immature female measured only 16 mm. long). The breadth of the body is 0.22 mm.

COBB's formula is : $\frac{—, 0.54, 1.7, 2.5, t^*,}{—, 0.8, 0.88, 0.84, 0.8,}$

The head end [plate I, fig. 2] is somewhat bulbous, and has the shape of a short cone slightly flattened at the apex—which is the position of the oral orifice. On the rim of the slightly flattened area are four minute papillae. The oral orifice is placed centrally—no buccal appendages can be made out. The buccal cavity is continued backwards into a thick-walled narrow-lumened oesophagus, which is 0.45 mm. long, bulbous posteriorly, and distinctly marked off by a constriction from the rest of the alimentary tract. What appears to be the nerve collar or commissure crosses the oesophagus at a distance of about one quarter of its length from the anterior end. The gut is continued almost straight down the length of the worm, curving only from side to side, and ending at the terminally placed anus. The position of the anal orifice is indicated by a depression placed slightly subterminally. The tail end does not taper, but is slightly swollen at the extreme end, which is very abruptly rounded off. (Plate I, fig. 3). The vaginal orifice is situated at a distance of 0.7 mm. from the anterior end, and is placed at the centre of a small conical papilla. Two minute pre- and two post-vaginal spines can be made out (there may be six in all). The vagina which has thick muscular walls is directed generally backwards, but according to the state of engorgement of the uterus it may first go a little backwards and then

* t denotes that the position of the anus is terminal.

make a loop forwards before turning; it coils backwards to a point about one-quarter down the length of the worm and receives the two horns of the uterus. In ripe specimens the vagina is seen packed with numerous outstretched embryos arranged longitudinally. The two uterine horns make many longitudinal coils and twists round each other, which may extend up as far as the junction of the oesophagus and intestine and backwards to the posterior end of the worm. Near their termination they narrow considerably and end in long blunt nodular extremities in the posterior quarter of the worm. In the mature worm the contents are first granular in the narrowed terminal portion; the granules increasing in size further on until distinct ova are made out. Beyond this they contain embryos coiled up in the vitelline membranes which, when the embryos have straightened themselves out, are seen to form the embryonic sheaths. In some of our specimens many embryos enveloped in their characteristic sheaths have escaped. These and the ova are found to have the following measurements:—

Length of ovum containing coiled up embryo	36 μ
Breadth	23 μ
Length of freshly hatched embryo	76.5 μ
Breadth	8.2 μ

The male is much smaller than the female. It is found in similar positions, and in general characters resembles the female, although it is shorter and thinner: it is characterised in preserved specimens by the strongly incurved tail, which makes two almost complete turns. Its average length is 7.5 mm., its breadth 0.15 mm.

$$\text{COBB's formula: } \frac{1.02, 4.07, 50, 1}{1.63, 1.63, 1.02,}$$

The head end (plate I, fig. 4) is similar in shape to that of the female. The length of the oesophagus is 0.32 mm. There is a distinct cardiac constriction. The anal orifice appears to be placed, not exactly terminal, but rather on the ventral surface. There are three pairs of pre-anal and one pair of post-anal papillae; the posterior two of the pre-anal series are larger, and are united by low ridges with the corresponding papillae of the opposite side. There are two spicules of unequal length—not extruded in our specimens. The tail end (plate I, fig. 5) does not taper, the extremity somewhat resembles that of the female, except that dorsally it is not so abruptly rounded off.

The embryo. The habitat of the embryo seemed to be essentially the lymph. In the process of the preparation of our specimens, it was often observed that in those made from the blood of the claws and legs by puncture of a small blood-vessel, one only, out of many slides, was occasionally found to contain very few embryos; many contained none at all. Moreover, we never found any embryos in the heart's

blood. On more careful examination it was found that the claws appeared somewhat oedematous, and by careful manipulation we were able to obtain specimens of the serous fluid, which contained large numbers of the embryos.

The embryo (plate I, fig. 1) in the fresh condition as seen in lymph and some blood preparations was 84.7μ long. The breadth of the sheath of the embryo, 12.78μ ; of the embryo itself inside its sheath, 7.9μ . When fresh the embryos exhibited a slow sinuous progressive movement: while, inside the sheath they were much more active. The two ends of the worm continually moved about, so that the tips seemed always in contact with the inner surface of the bluntly conical end of the sheath—the ends never being observed retracted from the sheath. This movement of the extremities inside the sheath, which appears a little too short for the embryo, causes the body of the embryo to be thrown into two curves, the sheath crinkling a little opposite the concavities of the curves. Ecdysis was not observed.

Both extremities of the embryo are bluntly rounded. At the anterior extremity is a short stout conical papilla from the apex of which projects a short thick spine which is always closely applied to the inner surface of the rounded end of the sheath. There is no prepuce, but a distinct ridge marks off the body from the papilla: neither spine nor papilla was observed to be withdrawn. Under high powers a central line appears to run down from the papilla into the body. The anterior portion of the body of the embryo tapers very slightly. The contents are finely granular, a larger more refractile granule appearing at a point at about a quarter of the length from the posterior end. At this end the worm has a short rather broad, highly refractile tubercle which is always in contact with the sheath, and moves from side to side along the concavity of the end of the sheath.

In fixed and stained specimens, in all of which the embryo is found shrunk in various degrees inside its capsule, the length varies from 75 to 84.7μ . The nuclei of the very small cells are evident, but indications of V-shaped or other shaped spots are very indefinite and irregular.

***Filaria spiralis avium.* Nov. Sp.**

Definitive hosts.—*Hyphantornis aurantius.*

Cyanomitra reichenbachi.

Muscicapidae. Sp. dub.

Pyenonotus barbatus.

Sitagra brachyptera.

Vidua principalis.

Cinnyris fuliginosa.

Cypselus affinis.

Site. The adult worms were always found in swellings about the feet and ankles of these birds. The infected birds were easy to detect by the presence of small, soft, subcutaneous tumours in these positions; the skin over these tumours was stretched, and the superficial veins appeared dilated.

The small nodules occurred in various positions: for example in one bird—on the right foot, a tumour on the upper surface of the second phalanx of the first toe, and in a similar position on the second toe; a third on the under surface of terminal phalanx of the fourth toe; another over the distal end of tarsus. In the left foot—a large swelling under the distal end of the tarsus; two on the first toe, one at its extreme base on the under surface, the other on the under surface of the terminal phalanx; and one on the lateral outer surface of the fourth toe. In another bird, one tumour was found higher up, in the tarsus among the tendons; others just at the base of the claws, under the insertion of the flexor tendons.

The worms occupied cysts in the positions mentioned, from two to ten worms in each cyst, which seemed to be intimately connected with the sheaths of the tendons. The worms were coiled together inside the cyst; the whole clump of them being easily turned out on slitting up the tumour, with a mass of yellow coloured jelly-like fluid, which surrounded the worms.

The worms in the cysts varied in colour from pale yellow to brown, the younger worms being generally brown. Some were considerably larger than others. They have a decidedly corkscrew shape, the screw having two to four turns according to the length of the worm. Introduced into normal salt solution the worms retained their corkscrew shape and moved for some time with a sluggish corkscrew motion. The shape is kept in preserved specimens. The screw of both male and female worms is a right-handed one, these facilitating the arrangement of a large number into the smallest space.

The female:—The total length varies from 4·4 to 8·4 mm., the central breadth about 0·34 mm. The worm makes three or four coils.

$$\text{COBB's formula: } \frac{\text{---}, 1\cdot8, 10\cdot2, 4\cdot3, 99\cdot2}{\text{---}, 1\cdot2, 2\cdot8, 1\cdot7, 0\cdot94}$$

The anterior and posterior portions of the worms beyond the beginning and end of the spiral are somewhat flattened; the anterior portion is longer than the posterior, and more sharply pointed. The cuticle is thick, transparent, and yellowish in colour: over the extreme ends it is thin. Laterally in the anterior portion the cuticle is thickened, the two lateral thickenings being continued down throughout the length of the worm, so that in optical sections of the convexities of the spirals they appear as knobs, of the concavities as thickenings.

The anterior end of the worm (plate II, fig. 2) which is tapered from the point of junction with the spiral proper, is rounded; there is a slight narrowing for a neck. The position of the oral orifice is marked as a small dent in the cuticle. No

papillae nor tubercles are evident. The long oesophagus, extending down beyond the vaginal orifice, is about 0.75 mm. long, and bears a narrow bulb marked off from the intestine by a slight constriction often hidden by the vagina. The gut extends along the whole length of the worm, and terminates at the anus on the ventral surface just in front of the posterior extremity of the worm. The anal orifice is surrounded by five delicate lips giving a rosette appearance. Side view the position of the orifice is marked by a slight baying (plate II, figs. 3 and 4). The vulva is at a distance of about 0.33 mm. from the head end, and appears to open ventrolaterally. The vagina runs, for a short way, directly backwards, makes a coil towards the head end, and runs down. The first portion is very thick-walled with small lumen; beyond this the walls get thinner, and the lumen is seen packed with stretched-out embryos. The vagina receives the two horns of the uterus, which coil and twist round each other, and extend to the posterior end of the worm. Each horn has muscular walls at its entrance into the vagina; the muscular walls get thinner and the lumen narrows somewhat in diameter until a kind of neck is reached following a distinct bulbous swelling, in the region of which the muscular walls are much thicker, forming a sort of 'pylorus.' Beyond this the tube again narrows, the walls are very thick, so that only a narrow lumen is apparent. At the junction of this thick-walled tube (oviduct) with the bulbous swelling (uterus), the former projects into the cavity of the latter to form a papilla with an opening at its centre. Beyond this thick-walled oviduct is the ovary—a long wider thin-walled portion, which further on narrows considerably, becoming cord-like, and ends in a terminal bulb, immediately in front of which is a small swelling. The length of the ovary is about 1.7 mm.—of the oviduct 0.9 mm. The total length from vulva to the end of the ovary is about 2.6 mm.

Mature ova—spherical cells having large rounded nuclei and distinct nucleoli—are found in the large dilated proximal portion of the ovary. The narrow-lumened oviduct is empty. The cavity of the uterus near its junction with the oviduct, and for some distance down contains innumerable spermatozoa surrounding several ova. Beyond this, the uterus contains ova in all stages of development.*

Length of ovum containing embryo 39 μ .

Breadth " " " 27 μ .

Length of embryo with its sheath 236 μ .

Breadth " " " 5 to 6 μ .

The male is similar to the female in appearance but considerably smaller; it makes two or three spirals. The tail end has its tip curled ventrally. Length of worm 3.4 to 3.7 mm.; breadth 0.2 to 0.3 mm.

COBB's formula $\frac{—, 2.3, 15.7, 50, 98.6}{—, 1.9, 3.8, 4.6, 1.5}$;

*A more detailed account of the histology of the reproductive and other systems of these worms will form the subject of a subsequent article.

The anterior end resembles that of the female but is smaller. The oesophagus is 0.57 mm. long (in one very transparent specimen only, a distinct cardiac constriction could be made out). The anal orifice is at a point 0.08 mm. from the tip of the tail (plate III, figs. 1 and 2). Four pre-anal and three post-anal papillae on each side could be made out; the two last post-anal being very small. The genital orifice is in the median line at the apex of a slight raised prominence. On each side of this prominence are two cuticular expansions, bearing the papillae and forming continuations of the lateral cuticular ridges. There are two curved unequal retractile spicules, the ventral of which, shorter than the dorsal, appears to be hollowed out on its dorsal surface for the latter's reception. The dorsal spicule is rod shaped and ends in a round knob. The other seems to widen at its deeper end and bends round the sides of the dorsal. The spermatic canal runs up the worm from the neighbourhood of the base of the spicules as a single narrow tube which soon widens to fill up almost the whole of the body cavity. A short distance from the head it becomes somewhat narrower, and ends after making a few turns in this region.

The embryos are found in large numbers in the peripheral and in the heart's blood. They have a sheath which is a long narrow cylinder with rounded ends. In fresh specimens the embryos exhibit a simple snake-like lashing movement, progressing forwards and backwards, and also a backward and forward motion inside the sheath. Some were seen to coil themselves up closely. The worm with its sheath (plate XIII, fig. 2) has a uniform thickness, except at the posterior end where it suddenly diminishes into a wall-marked 'tail.' The length of the worm inside the sheath was 208.6 μ , breadth 1.7 μ . In the living specimens two longitudinal lines of fine refractile granules can be observed, one about the junction of the anterior and middle thirds, the other about the junction of the middle and posterior thirds. The head end is rounded, no definite prepuce nor spine could be made out, beyond a highly refractile 'glans'-like tip. At the tail end the width of the worm suddenly diminishes at a distance of about 6.5 μ from the extreme tip to about 2 μ . The tail comes off eccentrically.

In stained specimens (plate III, fig. 5) no characteristic position is acquired. The average length of the worm, which varies considerably, is 216.8 μ . The column of small nucleated cells forming the body, at the head end is bayed out so that the end appears bifid. Five 'spots' (V- or otherwise-shaped) can be made out; their numbers however varies from three to five.

On examination of a number of embryos the distances of the spots from the end of the worm relatively to its length proves to be fairly constant, and we shall adopt throughout this work a method similar to COBB's to indicate their positions; expressing the distances of the middle of the spots from the anterior end in percentages of the total length:

1. A transverse slit: distance 24.5 per cent. of length; sometimes not seen.

2. A clear sometimes lateral, sometimes transverse spot ; distance 35.3 ; constant.
3. A long space in which the nuclei are loosely arranged, often anteriorly and posteriorly ending in a clear space, with the nuclei more densely arranged in between ; distance 66.7 ; constant.
4. A small spot only occasionally seen, at 76 ; sometimes merges into the third spot.
5. A very small lateral spot sometimes absent ; distance 89.5.

Filaria fusiformis avium. Nov. Spec.

Definitive hosts : *Spermestus cucullatus*.

Hyphantornis. Sp. incert.

Hyphantornis aurantus.

Sites. The adult forms were found in the mesentery at the under surface of the liver and in the lung. The collection comprises two males, four females, and three immature worms : of which two were found in one bird ; a single one in another ; another single one in a bird whose blood contained also embryos of *F. spiralis* and *F. opobensis* ; the others in a fourth.

The female is a long whitish worm, tapering gradually for some considerable distance at each end. The length varies from 15.8 to 25.5 mm. ; breadth about 0.28 mm.

$$\text{COBB's formula : } \frac{—, 0.67, —, 1.6, —}{—, 0.27, —, 0.33, —}$$

The cuticle is thin, smooth and transparent, somewhat thickened at the head end. The worms are all very opaque, so that but little of the internal anatomy can be made out in preserved specimens. At the anterior end (plate IV, fig. 2) which tapers to a breadth of 0.05 mm., is a slight appearance of a somewhat narrower neck. The oral orifice is terminal ; no papillae nor spines apparent. The vulva is at a distance of about 0.43 mm. from the anterior end. The anus is terminal. The posterior end also tapers considerably to 0.05 mm., and is then abruptly rounded off (plate IV, fig. 3). The ovum measures 27.7μ by 24.7μ , and the length of the embryo inside the sheath in the preserved condition is 120μ . The male, one specimen only of which is suitable for description, is 11.8 mm. long, 0.16 mm. broad.

$$\text{COBB's formula : } \frac{—, —, —, 50, 0.88}{—, —, —, 13.8, 0.47}$$

The cuticle shows no obvious striation. The head end resembles that of the female in shape ; it is provided with four small tubercles round the oral orifice. The posterior third of the worm tapers and coils, the coiling increasing towards the end of the tail. The anal orifice opens at a distance of about 0.14 mm. from the tip of the tail. There are two unequal spicules. The specimen is too opaque to make out any further internal anatomy.

The *embryos* are found in the peripheral and in the heart's blood. Its length in the fresh condition is 117μ , breadth, 3μ . It has a very marked sheath which can often be seen trailing in front or behind the worm in the fresh specimen (plate XIII, fig. 3). The embryos move backwards or forwards with snaky movements, and also rush very energetically forwards and backwards inside their sheaths. The head end is rounded and has a six lipped prepuce through which a conical papilla can be observed to be protruded; this bears at its apex a fine projecting spine. The papilla and its spine can be retracted within its sheath—an action very actively performed in fresh microscopical preparations. The body contents appear finely granular; at the junction of the anterior and middle thirds is a highly refractile spot. The tail end tapers very slightly and ends bluntly rounded. In stained specimens (plate IV, fig. 4), the length of the embryo is $86\cdot0\mu$; the length of the sheath beyond the worm proper both anteriorly and posteriorly varies enormously. The head end shews generally only a looseness in the arrangement of the small cells—sometimes a baying.

- Spots*.—1. A narrow transverse slit—fairly constant; distance $28\cdot6$.
 2. A lateral rounded bay—not always present; distance $40\cdot7$.
 3. A clear band across the worm—constant; distance $69\cdot2$.
 4. A small lateral slit—only occasionally seen; distance $90\cdot0$.

Spots 2 and 4 are always on the same side of the worm.

Filaria spiralis avium major. Nov. Sp.

Definitive hosts: *Hyphantornis*. Sp. incert.

Sitagra brachyptera.

Hyphantornis aurantius.

Site. In *Sitagra brachyptera* the adult worms were found in a thick walled cyst on the right leg situated deeply under the tendons on the bone. The cyst contained one small (male) and two large (female) worms. This bird also contained embryos and adults of *F. spiralis*. Although similar in appearance and site the two worms are quite distinct, the female of *F. spiralis major* being three or four times the length of the female of *F. spiralis*—and moreover they have different embryos, a fact conclusively demonstrated by the rupture of the uterus in each case and the examination of the contained embryos.

The female, spiral in form, has nine turns. The spiral is right-handed. The total length is $17\cdot3$ mm., the central breadth $0\cdot43$ mm.

$$\text{COBB's formula: } \frac{\text{---}, \text{---}, \text{---}, 2\cdot4, \text{---}}{\text{---}, \text{---}, \text{---}, 0\cdot64\text{---}}$$

Similarly to *F. Spiralis*, there is an anterior and posterior portion beyond the spiral, the anterior of which is the longer. The cuticle at the anterior end is thin, but thicker at the posterior end. Laterally the cuticle is thickened, and along the

convexities of the worm is seen to be distinctly striated transversely. The outer lateral ridge bears a number of flattened transparent nodules, the distribution of which seems to be irregular, in places appearing to be grouped into twos, threes, or fours. The striations of the cuticle become spread out in the nodules. The striations are less distinct along the other lateral border of the worm. The anterior portion (plate V, fig. 2) tapers considerably; its extremity is rounded; the oral orifice is central and terminal—no papillae or other appendages could be made out. The anus is in a position similar to that of *F. spiralis* (plate V, fig 3). The vagina opens at a distance of 0.45 mm. from the anterior end. The opacity of the worms makes it difficult to observe further the internal anatomy. The nodulated cuticle is very characteristic.

The male is much shorter and thinner than the female and has six coils. The tail end is markedly incurved. The length of the worm is 9.0 mm., breadth 0.125 mm.

$$\text{COBB'S formula } \frac{-, -, 3.4, 50, 99.2}{, -, 0.93, 1.4, 0.46}$$

The cuticle is ridged, knobbed, and striated, similarly to that of the female. Beneath the lateral ridges in the musculo-cutaneous structure is a dark brown granular pigmented layer: the pigment, apparently intracellular, is regularly interrupted by what appear to be large unpigmented nuclei. The head end (plate V, fig. 4) is similar to that of the female. The oesophageal bulb is very indistinctly marked. The anal aperture is at a distance of 0.058 mm. from the tip of the tail, and is at the centre of a low flat papilla. Three pre-anal, and two post-anal papillae could be made out on each side, the former being very small and close together, almost continuous with one another. There are two unequal spicules, their terminal extremities have a rosette appearance. The origin of the reproductive tube can be seen to commence as a thin single tube coiled about the neighbourhood of the commencement of the intestine, which increases in size to fill almost completely the whole body cavity up to the last coil of the worm where the tube becomes thinner and its walls more muscular till it ends at the rosette horns of the spicules. The tail end differs from that of *F. spiralis* having no expansions of the lateral cuticular ridges, these disappearing altogether as the tail is reached. The tip is bluntly rounded off on the dorsal surface. (Plate V, fig. 5).

The *embryos* are found in peripheral and central blood. In the fresh condition they measure 141.7 μ in length, 6.5 μ in breadth. They exhibit forward and backward sinuous progressive movements. They have a well marked thick cuticle which shews distinct transverse striations (plate XIII, fig. 4). They taper very slightly towards the anterior end, very abruptly posteriorly, so that this end resembles in shape the point of a wire nail. The head end is bluntly rounded and has a small clear area—no prepuce nor spine could be made out. At the tail end the cuticle is well seen.

In stained specimens (plate VI, fig. 1) the cuticle is also well marked. The worm measures $119.3\ \mu$ in length. The embryos on fixing take up no characteristic position. At the head end the cell column is bifid. Four 'spots' are generally made out:

1. Slit like, at a distance of 29.2 per cent. of total length.
2. A small lateral bay; distance 42.6.
3. An oval-shaped spot occupying the breadth of the worm, containing only a few small nuclei; distance 64.4.
4. A small lateral break; distance 89.9.

The two lateral spots (2 and 4) are on the same side of the worm. All the spots are constant, but the first and second are sometimes badly marked.

Filaria falciformis. Nov. Sp.

Definitive Host: *Cinnyris fuliginosa*.

Site. The subcutaneous tissue of the back of the head, dorsum of wing, root of neck, and leg.

In one bird of this species three males and two females were found; in the second, one male and one female (with adult forms of *F. bibulbosa*); in another, one male and three females (also with some adults of *F. bibulbosa*), and in the fourth, one male and three females.

The female varies in length from 20.3 to 29.4 mm.; its breadth is about 0.23 mm.

$$\text{COBB'S formula: } \frac{—, 0.58, 0.76, 28.7, 98.9}{—, 0.62, 0.62, 0.69, 0.31}$$

It is creamy white in colour; a long thin worm with a slightly curved tail end. The transversely striated cuticle is finely ridged, the ridging disappearing near the head end. The head end (plate VII, fig. 2) is bluntly rounded, and tapers slightly. The mouth is terminal, and is simple, bearing no papillae. The oesophagus is a straight thick-walled tube, and has no bulbous ending; the intestine commences suddenly as a broad tube full of dark granular substance, with here and there large irregularly angular masses of orange-coloured material. The position of the anus is on an average at 0.38 mm. from the posterior end; the orifice is at the summit of a low flattened papilla. No anal papillae can be made out. The body rapidly tapers beyond the anal aperture and ends in a cone-shaped portion 0.047 mm. across at its base (plate VII, fig. 3). The vulva is situated at 0.774 mm. from the head end of the worm, at the apex of a nipple-shaped papilla. The vagina courses down the worm, or may make a twist upon itself: it divides at about 1.5 mm. from its orifice into the two uterine horns, which, coiling many times on themselves, occupy almost the whole of the coelomic cavity. They end in a somewhat similar manner to that described under *F. spiralis*, except that no evidence of the existence of a 'pylorus' can be made out, and moreover, the extreme end is not bulbous.

The ovum, containing a coiled-up embryo, measures $27.7\ \mu$ by $19.5\ \mu$. The embryo is $112\ \mu$ long by $4.2\ \mu$ wide.

The male is much shorter than the female, and very active when freshly introduced into normal salt solution. It is very slender, and has a well marked incurved tail. It measures 11.6 to 14.8 mm. long, and its breadth averages 0.136 mm.

$$\text{COBB's formula: } \frac{—, 1.29, 2.53, 50, 98.8}{—, 1.04, 1.04, 1.38, 0.69}$$

The cuticle is striated and ridged as in the female. The head end (plate VII, fig. 4) is also similar to that of the female. The mouth is terminal and simple: there are no appendages.

The length of the oesophagus is 0.03 mm., there is no bulb. The anal orifice is at 0.149 mm. from the tip of the tail. The reproductive system consists of a single tube commencing thin, gradually increasing in width and occupying the greater part of the body cavity; it seems to end in connection with the bases of the spicules. The tip of the tail (plate VII, fig. 5) has four papillae, two of which are terminal so that the end appears bifid: the other two are placed dorso-ventrally to these and are much smaller. In front of these on the ventral surface are four papillae arranged in two pairs; while still further forward are two other post-anal papillae on each side; no pre-anal papillae could be made out. There are two unequal spicules, in many of the specimens extruded. (Plate VII, fig. 5, and plate VIII, fig. 1). The orifice ($16.3\ \mu$ across) through which they protrude resembles a wide crater with sharply defined edges at the summit of a low cone. The dorsal spicule seems to widen at its base and embrace the ventral; this spicule is pointed.

The *embryos* are found in the peripheral and central blood. The length varies very much in the fresh condition from 91 to $107.5\ \mu$: the embryo can be seen stretching itself considerably. Breadth $3.26\ \mu$. There is no sheath (plate XIII, fig. 5). The head end is blunt and there is some differentiation into a small papillae bearing a short stumpy spine. The tail end tapers a little and ends bluntly. The contents of the body of the worm are somewhat closely granular. In the fresh condition this embryo is characterised by the possession of a very distinct oval very highly refractile globule behind the middle point of the worm, almost at the junction of the middle and posterior thirds.

In stained specimens (plate VIII, fig. 2) the embryos only measure $87.3\ \mu$ on an average. The anterior bay in the column of cells at the head end is well marked.

The cells in these specimens appear to be loosely arranged. Four spots can sometimes be seen, but three of them are extremely variable. Sometimes one only, sometimes two, three, or four are present. One is constant—the third, and is a distinguishing feature of this embryo.

1. A small slit at a distance of 25.4 per cent. of the length of the worm.
2. A **V** which may extend across the breadth of the worm, distance 34.4 .

3. A band across the worm about 4μ wide—distance 62.2 (corresponds in position to that of the highly refractile granule seen in fresh specimens.
4. A slight lateral bay, distance 83.7.

Filaria bibulbosa. Nov. Sp.

Definitive hosts : *Cinnyris fuliginosa*.

Sites. Subcutaneously, in various positions. The worms generally occurred in pairs, male and female together. Our collection contains a single male and female in one position from one bird, and a single male from another position : this bird also contained adults. Two females were found in one bird of the same species, which also contain *F. spiralis*.

The female is a long, thin, whitish, smooth worm, both ends of which are bulbous. Its length varies from 20.7 to 22.7 mm.; its breadth is about 0.17 mm.

$$\text{COBB's formula : } \frac{\text{—, } 0.09, 0.10, 0.29, t}{\text{—, } 0.05, 0.05, 0.05, 0.06}$$

The cuticle is somewhat thick, smooth, not striated. There is a slight narrowing for a neck (0.13 mm. wide) separating off the bulbous head end (0.17 mm.) (plate VIII, fig. 4). The mouth is terminal; no papillae nor other appendages discernible. The oesophagus is straight, has no bulb; length 0.25 mm. The anus (plate VIII, fig. 5) is terminal and central, and is surrounded by four small lips. The vulva is 0.65 mm. from the anterior end : it is situated on a low conical papilla. The vagina is directed backwards, but may coil forwards as in other filariae. The uterine horns resemble those of others previously described. The two extreme ends are bulbous; no 'pylorus' could be made out.

The male is smaller and thinner than the female, otherwise similar in appearance; the tail end is not incurved. Length 8.6 mm.; breadth 0.09 mm.

$$\text{COBB's formula : } \frac{\text{—, } 1.86, 2.26, 50, t}{\text{—, } 0.93, 0.93, 0.93, 0.93}$$

Width of head 0.1 : of neck 0.07 mm. The head end, mouth and oesophagus are similar to those parts in the female (plate IX, fig. 1). Posteriorly the dorsal surface is rounded off to meet the ventral surface at an angle, at which the anal orifice is situated (plate IX, fig. 2). The region round the aperture is slightly flattened. In both of the specimens in our possession, one of the spicules of this worm is extruded through the orifice. It is curved and sharply pointed; the other appears to ensheath the former. Only one small (probably a pair) post-anal papilla can be discerned. The reproductive tube is similar to that of the other filariae.

The *embryos* are found in the blood, both peripheral and central. They have no sheath : they are capable of progression in both directions, exhibiting sinuous movements (plate XIII, fig. 6). Length 117.4 μ , breadth 4.9 μ . The body is plain

and appears structureless. The front end is bluntly rounded; and has no prepuce nor spine: the tail end tapers gradually from almost the middle of the worm to the tip of the tail.

In stained specimens (plate IX, fig. 3) the embryos set in characteristic comma-like position. The embryos measure 97.8μ in length.

The head end does not show a baying, but simply a looseness in the cell arrangement. The following spots are always present:

1. A small central irregular clearing, at distance 22.5 per cent. of length.
2. A similar central irregularly shaped clearing, larger than the first, distance 33.5.
3. The largest spot, oval in shape, distance 60.7.
4. A tail spot, the second largest, well marked, oval, at 81.8

Filaria capsulata. Nov. Sp.

Definitive hosts: *Pyenonotus barbatus*.

Sitagra brachyptera.

Hyphantornis. Sp. incert.

Sites. In *Pyenonotus barbatus*, in the tissues between the oesophagus and spinal cord, were found three bundles which microscopically appeared to consist of a thin membranous capsule containing a worm or worms coiled up. These on dissection were found each to consist of a thin connective tissue capsule with two worms, a long one and a short one coiled up, in its interior.

In another bird of the same species were found five encysted worms between the oesophagus and spine; there was no free worm, but on dissection of one of these cysts the head of a worm was found to project about 1.0 mm. length out of the cyst. One of the cysts was very small; another large one contained a yellow coloured lightly mottled worm.

In still another bird of this species ten flattened masses were found in similar positions; they looked like bags of whitish jelly containing coiled worms. They were directly subcutaneous or on the muscle fascia with delicate fibrous tissue bands anchoring them to the tissues below, so as to permit of some movement but requiring dissection for removal. The positions in which they were found were:—one on the back of the head, three in the neck, two between the trachea and muscles of the spinal column, another at the base of the neck, one at the lower edge of the pectoral muscular mass laterally, two on the thigh.

Apparently each sack contains two worms; some of these were purposely torn across—characteristic ova and embryos issued from the ruptured uterus.

In *Sitagra brachyptera* in a single case the site was lower down the oesophagus—a cyst of yellowish colour was found between oesophagus and liver. This bird also contained embryos and adults of *F. spiralis* and *F. spiralis major*.

In the *Hyphantornis* a cyst occurred under the skin of the thigh and contained two worms.

The cyst (plate X, figs. 1 and 2) is a thin-walled delicate fibrous tissue capsule which is whitish in colour, almost transparent and closely applied to the worms which it contains. The worms are often difficult to separate entirely from the enveloping capsule. The cysts seem to contain no or extremely little fluid; each has always two worms, a male and a female. The colour of the contents varies from white to yellow, according to the colour of the contents of the intestine canal of the worms. The dimensions of the capsule vary: the largest was 5.7 by 3.4 mm., the smallest 1.8 by 1.2 mm.

The female worm, in one completely dissected-out specimen, measured 40.6 mm., breadth 0.44 mm.

$$\text{COBB's formula: } \frac{—, 0.98, 1.86, 1.26, 1}{—, 0.69, 0.86, 0.75—}$$

The cuticle is thin and smooth.

The head end (plate X, fig. 3) tapers somewhat, and is bluntly rounded. The mouth is terminal; there are no papillae nor other appendages. The oesophagus is straight, has no bulb; it is 0.54 mm. long, is light in colour, and indistinctly marked off from the darker intestinal tract. In the tract of many specimens are numerous bright orange-coloured round clumps of material—which give rise to the yellow colour of the worm. These clumps are irregularly shaped—some round and others angular. The tail end (plate X, fig. 4) is bluntly rounded; the anal orifice is terminal. The vulva is situated on a small conical papilla, 0.25 mm. from the anterior end. The vagina extends directly, or after one or two forward twists, down the body of the worm for 1.6 mm. distance from the orifice, where it receives the two uterine horns. These resemble those of the *Filaria* already described. There is a well marked 'pylorus' beyond which the tube is continued for 4.48 mm., to terminate in a blunt, slightly nodular end. The ova, containing coiled-up embryos, measure 26 μ by 19.5 μ . The embryos are 81 μ long.

The male in shape and appearance resembles the female, but is much smaller. Length 4.5 mm.; breadth 0.17 mm.

$$\text{COBB's formula: } \frac{—, 3.64, 9.26, 50, 97.6}{—, 2.64, 2.64, 3.80, 2.32}$$

The ends, both anterior (plate XI, figs. 1 and 2) and posterior (plate XI, figs. 1 and 2) are similar to those of the female. Width of head, 0.11 mm.; of tail end, 0.10 mm. Length of oesophagus, 0.39 mm. The anus is ventrally placed, a little in front of the posterior end of the worm. There are probably two spicules: one, sharply pointed, is extruded in some specimens; the other could be but very indistinctly made out.

The embryos occur in both central and peripheral blood. They have no sheath. Length in the fresh blood 94.5 μ , breadth 3.5 μ . The head end is slightly

tapered. The tail end tapers very gradually for the last third of the length of the worm and then at a distance of about $10\ \mu$ from the tip more rapidly, to end bluntly. The contents of the body of the worm are granular (plate XIV, fig. 7).

In stained specimens (plate X, fig. 5) the length of the embryo only averages $81.5\ \mu$. The column of cell nuclei at the head end appears abruptly broken off—there is some looseness of the cells here also.

The 'spots': generally two are seen, sometimes only one, which is constant.

1. A narrow slit or break in the cell column; distance 32.9 per cent. of whole length.
2. Oval in shape, occupies the whole breadth of the worm; distance 58.5 . This spot is constant.

***Filaria shekletoni*. Nov. Sp.**

Definitive hosts: *Cypselus affinis*.

Hyphantornis aurantus.

Site. In *Cypselus affinis* our only two specimens, both females, were found, one lying under the pericardium along the whole length of the heart; the other in the peritoneal cavity on the upper surface of the liver. On breaking one of these, numerous embryos, similar to those found in the heart's blood, emerged.

The female is white in colour, 12.5 mm. long, 0.29 mm. broad.

$$\text{COBB's formula: } \frac{—, 1.66, 4.22, 33.4, t}{—, 1.44, 2.11, 1.88, 0.55}$$

The body tapers towards the head end (plate VI, fig. 4) which is rounded and somewhat flattened dorso-ventrally. The oral orifice is terminal, and has no appendages. The oesophagus is straight, has no bulb, measures 0.5 mm. in length. The anal orifice is not quite central terminally, but is situated a little towards the ventral surface. The tail end (plate VI, fig. 5) is slightly curved and flattened. The vaginal orifice is at 0.42 mm. from the anterior end. The vagina and uterine tubes resemble those of filariae previously described. The egg measures $46.6\ \mu$ by $33\ \mu$. The length of the embryo $315\ \mu$.

The male is unknown.

The *embryo* in fresh blood specimens are very long, roughly measured to be about $360\ \mu$; it has very active lashing movements, but only slowly progressive. It has no sheath; the contents are somewhat coarsely granular; the head end is rounded, has no papilla nor spine; the tail end tapers gradually to a very fine point.

In stained specimens (plate VI, fig. 2) the length averages $235\ \mu$, breadth $6.4\ \mu$. There is a very slight transverse striation of the somewhat thick cuticle. The head end shews no baying in the column of cells, which here ends abruptly.

Four 'spots' can be observed—all of which are constant:—

1. A narrow slit; distance 22.0 per cent. of the length of the worm from anterior end.
2. A bright oval lateral bay; distance 29.3.
3. A long portion in the middle of the worm in which the cell nuclei are few in number and stain less distinctly than the rest of the worm. Its middle point is at a distance of 61.7. This is characteristic of the worm.
4. A lateral bay, similar to and on the same side as the second; distance 84.9.

SPECIES OF FILARIAE, THE EMBRYOS OF WHICH WERE FOUND, BUT NO ADULTS

Filaria serpentiformis

Definitive host : *Cinnyris fuliginosa*.

F. falciformis also occurred in the blood of this bird. The embryos were found in small numbers in the blood; but many were present in the lung juice, while only a very occasional one was seen in preparations of heart's blood.

In fresh specimens (plate XIV, fig. 8) the embryos measured 436μ ; breadth 6.2μ . They were very active, quickly coiling and uncoiling: only slightly progressive. The head end had no papilla nor spine, only a clear conical tip. Body contents granular, no distinctive spots. The tail end tapered gradually for about one-sixth of the length of the worm to a very fine point.

In stained specimens (plate XII, fig. 1) the length was 339μ . Head end, which is slightly tapered, is round, and for a distance of about 10μ from the tip shews no nuclear staining. A single narrow band-like or V-shaped spot only, at a distance 19.9 per cent. of total length of the worm from the anterior end can be made out. At the junction of the posterior and middle thirds is an indefinite area in which the stained nuclei are looser. The tail end consists of a single column of cells gradually diminishing in size.

This embryo resembles somewhat that of *F. shekletoni*, but it is much longer both in stained and fresh specimens and the arrangement of the 'spots' serves to distinguish them.

***Filaria opobensis*. Nov. Sp.**

Definitive hosts : *Hyphantornis aurantus*.

Hyphantornis. Sp. incert.

Stained specimens only obtained (plate XII, fig. 2). The length of the embryo varies considerably both in specimens of blood from different birds and in

specimens from the same bird. Average $43\ \mu$ (37.5 to $61\ \mu$). Breadth $6.6\ \mu$. The nuclei which are very small, stain very deeply. At the head end the column of nuclei breaks into two lines to form a 'bay.' The tail end tapers in the last sixth to about one-half of its width, and then terminates in a small bulbous end. There is a very thin cuticle.

'Spots.' These can be distinctly made out, only one is constant.

1. A small irregular transverse slit, at distance 25 per cent. of length of the worm.
2. A slight lateral bay at 33.8.
3. A band across the worm at distance 60.0. This is the constant spot and a characteristic of stained specimens of this embryo.

Filaria calabarensis. Nov. Sp.

Definitive host not yet identified.

The embryos were found in central and peripheral blood. In some blood specimens (plate XII, fig. 3) these embryos occurred alone; in others, from other birds, they occurred with *F. bibulbosa* and *F. falciformis*. They were present in five birds out of nine examined. Stained specimens only available for description. Length $163\ \mu$; breadth $4\ \mu$. No sheath, but the thin cuticle shows very slight striation.

The anterior extremity is rounded, and there is a 'bay'-shaped opening in the column of cells, which is thus bifid. The uniform width of the worm is maintained up to the position of the third 'spot' described below, where the worm begins to taper to a very fine point.

The following 'spots' are observed:—

1. A small anterior central irregular 'spot,' distance 24.2 per cent. of length from the anterior end.
2. A shallow lateral 'bay,' at 34.6.
3. The largest and most distinct of the 'spots,' roughly diamond-shaped, at 60.8.
4. A lateral break, occurring at the same side as the second, at 82.8.

A FILARIA, THE ADULT MALE OF WHICH ALONE WAS FOUND;
FEMALE AND EMBRYOS NOT OBSERVED

Filaria phoenicopteri. Nov. Sp.

Definitive host : *African Flamingo.*

The Flamingo had been skinned and cut into pieces before the time of our examination. Five male worms were found under the skin and on the muscle fascia; the examination of the available blood showed the absence of embryos.

The length of the worms averaged 13.4 mm., breadth 0.26 mm.

$$\text{COBB's formula : } \frac{\text{---}, 2.67, 13.3, 50, 99.6}{\text{---}, 1.87, 2.40, 2.13, 1.06}$$

The cuticle is thick, transparent, ridged, is thinner at the anterior end of the worm. The head end (plate XI, fig. 4) tapers somewhat to 0.25 mm. ; there is a slight indication of a neck 0.25 mm. across. Over the position of the buccal orifice, which is terminal and central, there is a slight flattening on the edge of which are four small tubercles. The oesophagus is very long, and marked off by a constriction from the intestinal tract, which is seen to course down the worm, curving from side to side to end at the anus a little in front of the extreme tip of the tail. The tail end (plate XI, fig. 5) tapers for a considerable distance, and is incurved. The extreme end is bluntly rounded. The anal orifice is wide, placed on a slightly raised papilla ; through the orifice in some specimens, the tip, in others about 50 μ length of a single sharply pointed spicule, projects.

The worms are too opaque for further details to be made out.

IV. HUMAN FILARIASIS

The species of the genus *Filaria* which are supposed to give rise to haematozoal embryos found in human blood are :—

1. *Filaria bancrofti*, COBBOLD; syn. *F. sanguinis hominis*, LEWIS; *F. nocturna*, MANSON.
2. *Filaria diurna*, MANSON.
3. *Filaria perstans*, MANSON.
4. *Filaria demarquaii*, MANSON.
5. *Filaria ozzardi*, MANSON.
6. *Filaria magalbäesi*, MANSON.
7. *Filaria loa*, GUYOT.

Filaria bancrofti

Historical. The embryo of this parasite was discovered by DEMARQUAY in 1863 in the chylous fluid from a case of dropsy of the tunica vaginalis, who came originally from Havana. WUCHERER, in 1866, found the embryos in the urine of several cases of tropical chyluria. In 1868 and following years, LEWIS, SALISBURY, CREVAUX, and COBBOLD observed the parasite in similar cases in or from Calcutta, the United States, Gaudaloupe, and Port Natal. In 1872 the history of the discovery of the life of this parasite entered a new phase, when LEWIS found that the embryos had their normal habitat in the blood of man. DA SILVA LIMA, CREVAUX, and MANSON established the identity of these blood filariae with those occurring in cases of chyluria and lymph scrotum in Brazil, the Antilles, and in China. In 1876 BANCROFT found an adult worm in an abscess in a lymphatic gland in the arm, and later four others in a hydrocele of the spermatic cord. Since then DA SILVA ARANJO, LEWIS, MANSON, and others have found adult worms in different sites. MANSON, studying the disease in China, observed a periodicity in the occurrence of the embryos of the parasite in the peripheral blood, and deduced therefrom the function of some blood sucking insect to play the part of intermediary host. In 1879 he demonstrated the life history of the parasite in the body of the mosquito, *Culex ciliaris*. As to how the parasite reached man again from the body of the mosquito several theories were advanced, until Low, in 1900, in sectioning some of MANSON's specimens of infected mosquitoes, observed the filariae in the proboscis: which discovery naturally leads to the inference that they are introduced at the time of puncture of the skin by the mosquito.

Description. The adult *Filaria bancrofti* is a long, hair-like, transparent nematode, three or four inches in length. Males and females often are found

together; sometimes there are found several in a bunch in cyst-like dilatations of the lymphatic vessels, sometimes they inhabit the larger lymphatic vessels. The female is the larger, both in length and thickness. The length varies from 88 to 155 mm., the breadth from 0.6 to 0.7 mm. We have been unable to obtain COBB's formula for this worm. The body is plain, tapering towards the rounded head end rather abruptly to a neck, which is about one-third the width of the body; beyond which it is enlarged somewhat. The cuticle is finely striated. The mouth is terminal, simple, 4μ in width. The tail end tapers and ends bluntly. The anus opens on the ventral surface at a distance of 0.13 mm. to 0.28 (according to the size of the specimen) from the posterior extremity, on the summit of a projection which resembles a bilobed papilla. At the extremity of the tail the cuticle presents a small depression, surrounded by two small lips. The vulva is situated at a distance of 1.26 mm. to 2.56 mm. (according to the size of the specimen) from the anterior end. The worm is ovi-viviparous. The ova measure 25μ to 38μ by 15μ .

The male has a length of about 83 mm., breadth 0.407 mm. The body is cylindrical, tapering gradually from the anterior to the posterior end. The tail is vine-tendrill like, the extreme end being sharply incurvating, making one or two spirals. The cuticle is delicately striated transversely. The anterior end is rounded, and not marked off by a neck from the rest of the body. The mouth is circular, simple, and terminal. The cloaca opens on the ventral surface at 0.11 mm. from the extremity. The tail end presents four pairs of pre-anal and four pairs of post-anal papillae, having a wide base. The oesophagus has a thick muscular wall, which gives it the appearance of a pharyngeal bulb: it is 0.99 mm. long, and is well marked off from the intestine. The genital tube is single. The cloaca gives exit to two unequal spicules.

The *embryos* measures from 270 to 340μ long by 7 to 11μ wide.

MANSON¹ describes the parasite and its movements thus:—‘In fresh blood, *F. nocturna* is seen to be a minute, transparent, colourless, snake-like organism which, without materially changing its position on the slide, wriggles about in a state of great activity, constantly agitating and displacing the corpuscles in its neighbourhood. At first the movements are so active that the anatomical features of the filaria cannot be made out. In the course of a few hours the movement slows down, and then one can see that the little worm is shaped like a snake or an eel—that is to say, it is a long, slender, cylindrical organism, having one extremity abruptly rounded off, the other for about one-fifth of its entire length gradually tapering to a fine point. . . . When examined with the low power, it appears to be structureless; with a high power, a certain amount of structure can, on close scrutiny, be made out. In the first place, it can be seen that the entire animal is enclosed in an exceedingly delicate, limp, structureless sack, in which it moves backwards and forwards. This sack or “sheath”

1. Manson, *Tropical Diseases*, London, 1900; p. 485.

as it is generally called, although closely applied to the body, is considerably longer than the worm it encloses, so that that part of the sack which for the time being is not occupied is collapsed, and trails after the head or tail or both, as the case may be. It can be seen also that about the posterior part of the middle third of the parasite there is what appears to be an irregular aggregation of granular matter which, by suitable staining, can be shown to be a viscous of some sort. This organ runs for some distance along the axis of the worm. Further, if higher power be used, a closely set, very delicate transverse striation can be detected in the musculo-cutaneous layer throughout the entire length of the animal. Besides this if carefully looked for at a point about one-fifth of the entire length of the organism backwards from the head end, a shining triangular **V**-shaped patch is always visible. What may be this **V**-spot is brought out by very light staining with dilute logwood. The dye brings out yet another spot, similar to the preceding, though very much smaller; this second spot is situated a short distance from the end of the tail. The former I have designated the **V**-spot; the latter, the "tail spot." . . . Staining with logwood also shows that the body of the little animal is principally composed of a column of closely packed, exceedingly minute cells enclosed in the transversely striated musculo-cutaneous cylinder; at all events, many nuclei are thereby rendered visible. Dr. Low has recently pointed out to me that the break seen in all stained specimens in the central column of nuclei occurs at a point slightly posterior to the anterior **V**-spot. This break can only be recognized in stained specimens. When the movements of the living filaria have almost ceased, by careful focussing it can be seen that the head end is constantly being covered and uncovered by a six-tipped or hooked and very delicate prepuce; and, moreover, one can sometimes see a short fang of extreme tenuity suddenly shot out from the uncovered extreme cephalic end and as suddenly retracted.'

In the above description in all its details, our observations of the embryos occurring in cases in Nigeria completely agree; but we think that the movements of the embryos in fresh microscopical preparations previous to the stage at which the anterior tip of the 'sheath' of the worm appears to become attached to the glass, have been overlooked. If preparations be made and examined directly, it will be seen that the embryos, for a short period only, exhibit a rapidly progressive movement across the field—so rapid at first that they can only with some difficulty be traced. This movement quickly ceases, the sheath of the embryo apparently becoming attached by its tip as described.

In stained specimens in our collection we have been able to distinguish the following spots, and their positions are indicated in a manner similar to that already used in describing the embryos of avian filariae—namely in percentages of the total length from the anterior end. The measurements have been made on a number of embryos, the percentages having been found to agree very closely in each. The average total length in stained specimens was 180.2μ .

1. An irregular transverse break, at about 21·5 per cent. of length. This is constant.
2. A V-shaped spot or a transverse irregular break at a distance of about 30 per cent. of the whole length from the anterior end. This is nearly always present.
3. Represents the central aggregation of fresh specimens: an area of varying length in which the cells are loosely arranged—distance 63. The point from which measurements were made was the middle point of this area. This is constant.
4. An irregular sometimes oval spot, often present at distance 85.
5. A small central bright spot, only occasional present at distance 91·5.

We propose here, before referring to the singular feature in the life of the embryo filaria known as 'filarial periodicity,' to describe briefly *F. diurna*.

Filaria diurna

MANSON¹ writes of this worm:—'I have twice encountered in negroes a blood worm with the same dimensions and anatomical characters, so far as these have been made out, as *F. nocturna*, but differing from this latter parasite, inasmuch as it comes into the blood during the day and disappears from it during the night. One of these patients came from Old Calabar, the other from the Congo. The periodicity observed by the parasite was thoroughly made out by prolonged observation in one of the cases. As the man was in good health at the time, and was observing ordinary habits as regards the hours of sleeping and waking, there can be little doubt that the parasite was not *F. nocturna*. Some years previously this patient had a *F. loa* in one of his eyes; it is just possible, therefore, that *F. diurna*, as I name this blood worm, is the embryonic form of the sexually mature *F. loa*. This is merely a conjecture. I have no further observations to support it; indeed, the negative results as far as finding filariae in the blood in four cases of *F. loa* which I have examined, are against it. Nothing is known about its life history or pathological significance. From recent observations I believe it to be very common (1 in 4) in certain districts on the lower Niger, where it seems to take the place among the natives that *F. perstans* holds among the Congo negroes.'

Our observations of a large number of cases of infection of what would be described as *F. diurna*, among natives from all parts of the west coast of Africa, verify the description of the blood filaria as given above by MANSON. In fact, absolutely no difference could be detected between this embryo and that of *F. nocturna*, either in fresh or in stained specimens. In stained specimens the characters and positions of the spots resemble closely those of *F. nocturna*.

1. Manson, *Tropical Diseases*. London, 1900. P. 532.

Filaria perstans.

The embryos of this worm are present in the peripheral blood both day and night. The parent forms have been described by DANIELLS, who found them in the connective tissues at the root of the mesentery, behind the abdominal aorta and beneath the pericardium. The male is smaller than the female. The body is smooth and devoid of markings.

DANIELLS' describes these worms, and compares their lengths and breadths with those of the adult forms of *F. bancrofti* and *F. magalhãesi*, thus:—

	<i>F. bancrofti</i>	<i>F. magalhãesi</i>	<i>F. perstans</i>
Length of female	95 mm.	155 mm.	70 to 80 mm.
Thickness „	0.2	0.66	0.12
Length of male	44	8	45
Thickness „	0.10	0.25	0.06

The neck is longer than in *F. bancrofti*: the mouth is very minute; no differentiation of the alimentary canal into oesophagus and intestine could be made out. The female tail curves for the last 0.3 to 0.4 mm. Anus 0.145 mm. from the tip of tail. The tip of the tail is 'mitred.' The embryos *in utero* are blunt-tailed, not sheathed.

The male is like the female with regard to the head end. Two perfect caudal ends were found. They were very much coiled, and had one spicule and two papillae.

The embryos measure on an average 200 μ long by 4.6 μ broad: but their dimensions vary over considerable range, the embryos possessing to a remarkable degree the power to elongate and shorten. They have no sheath. The body tapers gradually for two-thirds of its length towards the tail end which is truncated and abruptly rounded. On examination of the head with the high powers of the microscope, a fang is generally observed, in constant play, protruded and retracted. No prepuce is to be made out. The movements of the embryos are extremely active, in very fresh preparations it is almost impossible to follow them as they rapidly wriggle about between the corpuscles. Progressive movement continues for many hours.

In stained specimens the embryos of our collection on an average measure 89 μ . Four spots can be made out:—

1. A narrow irregular transverse band at distance 26.4 per cent. of total length from anterior end. Nearly always present.
2. A wider irregular transverse spot at a distance 36. Only occasionally present.
3. The largest of the spots, but not always present; an irregular transverse area at 63.2.
4. A very inconstant central bright speck at 83.2 distance.

1. Daniels, *British Medical Journal*, 1898, vol. I, p. 1011.

In the blood of one native, a court messenger at Degema, we found on very many occasions an embryo similar to that of *F. perstans* in its movements, general shape, and appearance, but longer (average in stained specimens $151\ \mu$). Four spots in stained specimens were made out, and were more distinctly marked than in the case of the ordinary *F. perstans*.

1. A constant narrow transverse band at a distance of 24.2 per cent. of total length from the anterior end.
2. A small lateral bay at distance 32.4. Fairly constant.
3. A distinct small area, in which the cells are loosely arranged, at distance of 61.2. This is only occasionally present.
4. A small bright spot, sometimes lateral, sometimes central, at 81.2.

Filaria demarquaii

The embryo only of this worm is known. It is thus described by MANSON¹ who observed it in specimens of blood from natives of St. Vincent, West Indies, in 10 out of 150 examined. 'It resembles *F. nocturna* and *F. diurna* so far as shape is concerned, but differs from them in size. I have had no opportunity of making trustworthy measurements of living specimens in suitably prepared slides, but judging from rough preparations, *F. demarquaii* appears to be rather more than half the size of *F. nocturna* and *F. diurna*. It is sharp-tailed, like these, but in addition to the size it differs from them inasmuch as it observes no periodicity, being present in the peripheral circulation both by day and by night, and, also, in not being enclosed in a sheath. Nothing is known of its life history, minute anatomy, or pathological bearings. Possibly it is the embryonic form of *F. magalhãesii*—also a tropical American blood parasite. I have recently met with apparently the same parasite in the blood of natives of St. Lucia, West Indies, where DR. GALGEY has still more recently shewn that either it, or a similar blood-worm, is very common. It is quite possible that the sharp-tailed filaria (*F. ozzardi*) of British Guiana is the same species. I have also found a minute, non-sheathed, sharp-tailed embryo filaria in the blood of natives of New Guinea, likewise closely resembling *F. demarquaii*. Whether these various embryos belong to one or to several species it is impossible to decide until the parental forms of each have been discovered and compared.'

Filaria ozzardi

A single adult female and a portion of the male found in the subperitoneal tissues in the anterior abdominal wall of an aboriginal Demerara Indian by DANIELLS,² whose blood contained nematode embryos similar to those to which

1. Manson, *Tropical Diseases*. London, 1900. P. 533.

2. Daniells, *British Medical Journal*, June 17, 1899.

MANSON originally gave the name of *F. ozzardi*, are believed to be the parent forms of these embryos. DANIELLS compares the dimensions and characters of these adults with those of *F. bancrofti* and *F. perstans*; the table we reproduce here :—

DESCRIPTION OF FILARIA OZZARDI EMBRYO

	<i>F. bancrofti</i>	<i>F. perstans</i>	<i>F. ozzardi</i>
	mm.	mm.	mm.
Length	85 to 90	70 to 80	81
Greatest thickness	0·20 to 0·26	0·120	0·210
Diameter of head	0·055	0·070	0·050
Diameter of neck	0·049	0·054	0·039
Distance from head—			
(1) Of vaginal outlet ...	0·710	0·600	0·710
(2) Of ovarian opening ...	0·920	?	0·850
Distance from tail of anal papilla ...	0·225	0·145	0·230
Termination of tail	Blunt, circular, not bulbous	Slightly bulbous : covered with thick- ened cuticle pro- longed into two tri- angular appendages	Bulbous cuticle, not thickened

The embryos. We have been able to obtain only a very short and imperfect description of the embryo. OZZARD¹ and DANIELLS² described two embryos occurring in the blood of the aboriginal Indians of British Guiana—one a blunt-tailed worm, which has since been identified as *F. perstans*; the other, ‘sharp-tailed, is about the size of *F. demarquaii* and similar in shape, but has no sheath.’ No periodicity was observed in either case. ‘The tail (of the sharp embryos) tapers slowly for a great length of the body to a fine and quite sharp point; the embryos arrange themselves often in figures of 8. The specimens are longer, and often in their thickest part broader than in the blunt tails. The arrangement of nuclei is clearer and more distinct, and the whole worm less deeply stained. The nuclei (of the tail end) are always arranged in single file for a considerable distance, and the terminal one has its long axis parallel to the long axis of the worm; while from this the body of the worm is continued for about 0·01 to 0·02 mm. to its termination free from nuclei. There are no nuclei at the cephalic extremity; the first ones seen are rod-shaped, with unstained spaces between, and at some little distance from the head is a gap (V-spot).

Filaria magalhaesi

The adult worms, which alone are known, are described by MAGALHAES³ as having been found lying in the left ventricle of a child at Rio de Janeiro. The two

1. Ozzard, *British Guiana Med. Annual*, 1897.

2. Daniells *British Guiana Med. Annual*, 1898.

3. Magalhaes, *Rio des Cursos Theoricos e Prat da Fac. de Med. de Rio Janeiro*, No. 3, An. III, 1896.

worms found were sexually mature. No examination of the blood had been made. The worms were cylindrical, capillary, and opalescent, white, uniform in thickness except where the body tapered towards the tail and at the club-shaped oral end; the swollen oesophagus was well marked off from the intestine. The mouth was simple, circular and unarmed, the cuticle marked with fine transverse striations. The female measured 15.5 mm. long by 0.7 thick, the male 8.3 mm. long by 0.4 mm. thick. The vulva was 2.56 mm. from the head end, at a point which divided the length of the worm in the proportion of 1 : 59. The tale of the male possessed four pairs of pre-anal and four pairs of post-anal papillae and two spicules, 0.17 mm. long. The tail made one and a half to two spirals. Nothing is known of its life history.

***Filaria loa*. Guyot**

This worm varies from 16 to 70 mm. in length, average 30 to 40.

The female of our collection measures 50.8 mm. in length, 0.57 mm. in breadth.

$$\text{COBB's formula : } \frac{\text{---}, \text{---}, \text{---}, 5.0, 99.6}{\text{---}, \text{---}, \text{---}, 1.1, 0.39}$$

Description. The worm is of uniform thickness throughout the whole of its length, except at the head end where it sharply tapers, and at the tail where for some distance in front of its extremity, the worm gradually tapers to less than half its breadth. The cuticle bears a large number of small rounded bosses apparently irregularly arranged as also described by MANSON¹ and others. The head end has the shape of a cone, with an abruptly flattened apex, at the centre of which is the small oral orifice : no buccal appendages are apparent. The specimen is too opaque for the oesophagus and its junction with the intestine to be made out. The vagina opens at a distance of 2.5 mm. from the anterior extremity. The tail end which tapers considerably, terminates in a short incurved portion (in our preserved specimen), on the concavity of which at a distance of 0.2 mm. from the extreme tip is seen the anal orifice at the summit of a low broad papilla. At the extreme end are two small fine tubercles. The ova, containing embryos, measure 35 μ by 25 μ ; the embryos measure 210 μ long.

Descriptions of two male specimens are given by MANSON.¹ 'Length, 25 to 30 mm., breadth, 0.30 mm. Thickness uniform except where it tapers at the head and tail. Mouth simple, no papillae nor armature. The tail end is sharply incurved and perhaps excavated ventrally; it is not spirally twisted. The tail is provided with well marked lateral alae. There are four well marked papillae on each side of the ventral surface of the tail. The three anterior papillae are pre-anal and large. They are closely approximated, stout and bulbous at the free end. The fourth is ad-anal or post-anal and is distinctly nearer the middle line and considerably

1. Manson, *Transactions of Ophthal. Soc.* London, 1895. *Case of Filaria loa*, by D. Argyll Robertson. Charles, *Sci. Mem. Medic. Officers, Army of India*; vol. vii., 1892, p. 51. Argyll Robertson, *Transactions of the Ophthalmological Society*. London, 1895. *Case of Filaria loa*.

smaller. The fifth is much smaller than the other, and is conical and sharp pointed. There are two slender, unequal spicules. The cuticle is not obviously straited but is dotted over with a number of widely scattered nearly hemispherical smooth bosses. No definite arrangement of these bosses could be made out. The large bosses are at the middle of the worm. The internal structure could not be made out.'

The *life history* of *F. loa* is quite unknown. MANSON suggests that it is the parent form of *F. diurna*.

Of the embryos, MANSON¹ says :—'The more mature embryos resemble in size and shape those of *F. nocturna* and *F. diurna*, but in consequence of the method of mounting it is impossible (speaking of the particular specimen under examination) to say if they are possessed of a sheath or not. If they are possessed of a sheath, I should say that they are practically indistinguishable from the parasites mentioned.' LEUCHART states that the embryos of *F. loa* 'are enclosed in thin egg shells, and bear a close resemblance to *F. sanguinis*, but are smaller (0.21 mm.)'

Our experience of the few cases of *F. loa* which we met with during the expedition accords with that of MANSON, in that an examination of the blood day and night did not reveal the presence of filaria embryos. We have, however, recently received a female specimen of *F. loa*, removed from the eye of a Kroo boy by Dr. A. H. HANLEY, medical officer at Opobo, Southern Nigeria. An examination of the blood showed the presence of embryos. We have counted the embryos on four slides taken at different hours of the day and night which were sent with the adult specimen.

At 10 a.m. the blood preparation contained seven embryos

„ 3 p.m.	„	„	„	nine	„
„ 9 p.m.	„	„	„	no	„
„ 11 p.m.	„	„	„	one	„

These figures point to an infection with *F. diurna*, but the examinations being so few, in the light of the results of examinations of other cases, a very definite opinion cannot be given.

Dr. HANLEY also sent a specimen of a male *F. loa* removed also from the eye of a Kroo boy whose blood contained no embryos. We were fortunate enough to obtain at Bonny a single female of this species for our collection, and on breaking the worm across after preservation in formalin, sheathed embryos very similar to those of *F. diurna* were extruded from the broken ends of the worm. These embryos, extruded by pressure from the body of the uterus of a formalin preserved specimen, measured 208.5 μ long on an average, and have a distinct sheath, in fact, they appear similar to the embryos of *F. nocturna*. In stained specimens they measure 199.6 μ long. (It must be noticed that these embryos had been fixed in the

1. Manson, *Trans. of Ophthalm. Soc.* London, 1895. *Case of Filaria loa*, by Argyll Robertson.

body of the uterus by the formalin in which they were preserved, whereas the embryos of *F. nocturna* described and measured were fixed in blood films by absolute alcohol).

The following 'spots' were made out :—

1. An oval or diamond shape central spot, at a distance of 24 per cent. of the length of the worm from the anterior end.
2. An indistinct lateral area containing scattered nuclei—distance 37·3.
3. A longer portion of the worm which stains badly, and in which the nuclei are irregularly scattered : sometimes it is divided into two portions, anterior and posterior. Because of the bad definition of this area, its position could not be ascertained exactly.
4. A small lateral bay, at a distance of 86·2.

Filaria nocturna, diurna and perstans.

Geographical distribution. HIRSCH¹ gives an interesting account of the distribution of elephantiasis throughout the world. 'In the Eastern Hemisphere the disease is endemic in many districts : the Southern regions of the Asiatic continents and islands, such as the coast of Arabia, many parts of India, Ceylon and the Malay Archipelago, some districts of further India and the Southern and South-Eastern coasts of China. In Syria and in Japan the disease is not so common. In India elephantiasis is specially frequent along the littoral of Lower Bengal ; along the littoral swamp of the Orissa. It is found also in Pondicherry and at a few places on the Coromandel Coast ; but most of all on the Malabar coast, especially in the districts of Travancore and Cochin. In the Deccan and in upper India it occurs much less frequently, although small endemic centres exist. In Ceylon the disease is common, more especially along the coast. In the East Indies, the Lampong district of Sumatra, Banka, the Nicobars, and the Phillipines, are the regions most severely affected ; the disease is less often seen in the other islands such as Java and Amboina. It occurs also in Penang and in Cochin China.

Certain of the islands of Polynesia are among the worst regions of the globe for elephantiasis : such as the northern part of New Caledonia, the Tonga, and Fiji groups, the Samoa group, Wallis Island, the Society Islands (especially Tahiti and Raiatea), and the Gambia group. It is less common in the Marquesas and in the Hawaiian Islands. In Australia as well as in New Zealand, it is not endemic. In Africa, Réunion and Mauritius, the Seychelles, Madagascar and Nossi-Bé, the Mozambique and Zanzibar coasts, the whole coast of Upper Guinea, including the Gaboon and Cameroons country, and the Benin Coast, Gold Coast, Spice Coast and Sierra Leone, as well as the Senegambia, the disease is endemic. In parts of Tunis, Algiers, and Egypt nearest the Mediterranean, and the swampy valleys of the interior

1. Hirsch, *Handbook of Geographical and Historical Pathology*. New Sydenham Society, 1886, vol. iii, q. 712.

of Abyssinia, the disease is met with. In the upper valley of the Nile (Nubia and the neighbouring countries of the negro) elephantiasis would seem to be unknown ; on the other hand there are accounts of its endemic occurrence at some places in the Greater Soudan, such as Bornou, Segu Sicroro, and Ogooué. Under the same circumstances of locality we find the disease widely endemic in the Western Hemisphere : as in the coast regions of New Granada, Venezuela, and Peru ; in those parts of Brazil that are mostly tropical in character. On the coast and marshy levels of Guiana ; in many islands of the West Indies such as the Barbadoes, Martinique, Guadaloupe, Trinidad, St. Vincent, and St. Bartholomew ; as well as on the Gulf Coast of the Central American States of Nicaragua, Costa Rica and Panama and of Mexico.

In Europe, in Greece it is very rarely met with ; it has been more frequently seen in Turkey ; in the south of France also, and in Lisbon and southern Spain it would appear to be relatively common ; but the patients may be in great part such as have acquired elephantiasis in the East.'

As to the demonstration of the presence of *F. nocturna* in the inhabitants of these countries, search for the adults and the microscopical examination of the blood for embryos has not yet been very extensive ; but in so far as observations go, the results roughly cover the same extensive distribution as that of elephantiasis. MANSON¹ has examined blood films from many parts of the world, including Old Calabar, the Lower Niger, Dahomey, Zanzibar, Mombasa, in Africa ; Madras, Cochin, Ceylon in Asia ; Samoa, Fiji, the Friendly Islands in Polynesia ; Georgetown, New Amsterdam, and the littoral of Demarara in British Guiana ; and the islands St. Vincent, St. Kitts and Montserrat, and Trinidad among the Islands of the West Indies.

Filaria diurna. As the presence of this blood parasite is not associated with any marked pathological lesion, the determination of its geographical distribution (necessitating the microscopical demonstration of the embryos in the blood, and of their characteristic diurnal periodicity), has not been so exactly nor so extensively made. In 1900, MANSON² states that he has twice encountered the embryos of *F. diurna*, once in a negro from Old Calabar, and another from the Congo ; and further, that from recent observations, he believes it to be very common (one in four) in certain districts on the lower Niger. This short account seems to be the whole of the present knowledge of the distribution of the *F. diurna* throughout the whole world, excepting the discovery of what, we think, must be taken as *F. diurna* in the Friendly Islands by THORPE³ ; this will be referred to again later.

Filaria perstans. For similar reasons as in the case of *F. diurna*—the absence of apparent pathological lesions and of the necessity of frequent daily microscopical

1. Manson, *Tropical Diseases*, London, 1900, p. 483.

2. Manson, *Tropical Diseases*, London, 1900, p. 532.

3. Thorpe, *British Medical Journal*, 1896, vol. ii, p. 922.

examinations of the blood—the geographical distribution of this parasite is but little known. Until recently it was believed to be confined to Africa, MANSON¹ stating that ‘this parasite is very common in the blood of natives of large districts in West Africa. I have found it in natives from Old Calabar and from the basin of the Congo, both in the coast natives and in those from the interior. DANIELLS informs us that he has found it in a native of British Central Africa residing on the East side of Lake Nyassa. In many parts of the endemic districts it occurred in about half of the population. Professor FIRKET, of Liege, has confirmed this observation as regards the Congo district. Sometimes it occurs along with *F. diurna* and *F. nocturna* in the same individual. I have never found it in West Indian negroes, nor in fact, in natives of any country except West Tropical Africa, and in the aborigines of Demerara. I have twice found it in Europeans who had resided in the Congo.’

OZZARD² and DANIELLS³ found many cases of *F. perstans* among the aboriginal Indians of Demerara—some 130 miles up the Demerara River, and also up the Berbice River. DANIELLS also discovered the adult forms of the worms among the aborigines of British Guiana.

OBSERVATIONS ON THE DISTRIBUTION OF THE BLOOD EMBRYOS AMONG WEST AFRICAN NATIVES

We had opportunities, during the sojourn of the expedition in Nigeria, of examining the blood of natives from all parts of the West Coast of Africa, from Sierra Leone at its Western extremity, as far as the Old Calabar district at the Eastern, and from the coast inland as far as the region of the kingdom of Sokoto some 500 miles in the northern direction, and as far as Yola on the Benue river easterly. Throughout the whole of this vast area, the natives appear to be infected with *F. nocturna*, *diurna* and *perstans*: and there can be no doubt that the distribution of these parasites will prove to be much more extensive in Africa, and probably throughout the tropical world, than is at present supposed. The native Kroo boys whom we examined both day and night, generally remain in a certain place for a period varying from a few months to a number of years, usually having left their native districts after reaching manhood, returning thither at intervals. As a large number of the others examined were prisoners, these had often remained the greater part of their lives in their own countries, and had been transported to the towns at which we met them, for confinement for political, criminal, and other offences.

From our notes of cases we have made the following table, illustrating the number of cases of pure and mixed infection throughout the district mentioned above. In the table, N.D. and P. represent *F. nocturna*, *diurna*, and *perstans* respectively; N.D., N.P., D.P., represent a double infection with *F. nocturna* and *diurna*, *nocturna*

1. Manson, *Tropical Diseases*. London, 1900. P. 536.

2. Ozzard, *British Guiana Medical Annual*, 1897.

3. Daniells, *British Guiana Medical Annual*, 1898.

and *perstans*, and *diurna* and *perstans* respectively, while N.D.P. indicates the triple infection. The diagnosis of the nature of the infection is based on the examination of the blood at twelve mid-day and twelve midnight, and a case was judged to be one of *F. nocturna* or *F. diurna*, according to the presence of the larger number of filariae at one or the other time; where the numbers were close the infection was noted as a mixed *nocturna* and *diurna*, although we are aware that this may not have represented the actual state of infection, as will be seen below in the paragraph on 'periodicity.'

TABLE I.

	No. examined	No. infected with						
		N.	D.	P.	N.D.	N.P.	D.P.	N.D.P.
NATIVES OF :—								
<i>Southern Nigeria</i> , including the Old Calabar and Cross River districts ; Bonny, Opobo and New Calabar districts ; Akwete district ; Brass, Wari, Sapele and Benin River districts, and the Lower Niger district extending as far as Idah ...	135	7	19	16	7	2	2	1
<i>Northern Nigeria</i> , including Lokoja and the regions of Sokoto, Kano, and the Benue River district ...	22	3	2	2	1	1
<i>Lagos and hinterland</i> ...	6	...	2
<i>Gold and Ivory Coasts</i> ...	4	1	...	1	1
<i>Kroo Coast</i> ...	40	3	1	1	2	...	1	1
<i>Other districts</i> , including Sierra Leone ; and a few natives whose native country was not ascertained ...	18	—	1	3	—	1	—	—
TOTALS ...	225	14	25	23	10	4	3	3

The following table shows the percentage of infected natives in towns having different sanitary conditions; for example Group I contains a number of towns and villages situated chiefly near the mangrove swamp, which are usually in a deplorable filthy condition; the natives of this group were found to be infected with haematozoal embryos to the extent of 50 per cent.; whilst Group II contains comparatively clean up-country towns in the region beyond the mangrove swamp. Group III are large coast towns.

TABLE II

	Number examined	N.	D.	P.	N.D.	N.P	D.P.	N.D.P.	Number Infected
GROUP I									
Old Calabar...	35	...	8	9	I	...	I	...	19
Bonny	11	...	I	...	I	2
Brass	11	4	4
Okrika	4	I	2	...	I	4
Opobo	5	2	I	3
Bugama	5	...	I	I	I	3
Degama Town	8	I	I	...	I	I	4
New Calabar	5	2	I	3
Abo...	4	I	I	2
	88	7	16	14	5	I	I	...	44
GROUP II									
Lokoja	5	I	I
Abonnema	4	0
Akwete	3	0
Azumine	2	0
Obuzo	2	...	I	I
Abutshi	4	0
Idah...	2	I	I
	22	I	I	...	I	3
GROUP III									
Cape Coast ...	3	I	...	I	2
Lagos	5	...	I	I
S. Leone	7	...	I	I
Accra	I	I	I
	16	I	2	I	I	5

Periodicity

In the case of *F. nocturna* MANSON and others have been able on several occasions to demonstrate a 'periodicity' in the life of the blood embryo. MANSON¹ thus describes the phenomenon:—'If under ordinary conditions of health and habit, the blood of a patient be examined during the day, the parasite is *rarely* seen, *or, if it be seen only one or two specimens at most* are encountered in a slide. It would be found, however, that as evening approaches, commencing about five or six o'clock, the filariae begin to enter the peripheral circulation in gradually increasing numbers. The swarm goes on increasing until about midnight, at which time it is no unusual thing to find as many as three hundred, or even six hundred, in every drop of blood. . . . After midnight the numbers begin gradually to decrease; by eight or nine o'clock in the morning the filariae have disappeared from the peripheral blood for the day. This diurnal periodicity is, under normal conditions, maintained with the utmost regularity for years. Should, however, as MACKENZIE has shown, a filarial patient be made to sleep during the day and remain awake at night, the periodicity is reversed; that is to say, the parasites come into the blood during the day and disappear from it during the night. It cannot be the sleeping state, as some have conjectured, that brings about this periodicity; for the ingress of the filariae into the peripheral blood commences three or four hours before the usual time for sleep, and the egress several hours before sleep is concluded, and this egress is not complete until several hours after the usual time of waking. . . . A recent opportunity has enabled me to ascertain that, during their diurnal temporary absence from the cutaneous circulation, the filariae retire principally to the larger arteries and to the lungs, where, during the day they may be found in enormous numbers.'

To illustrate this phenomenon of periodicity we give the following table re-constructed from data given by MANSON.²

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1. Manson, *Tropical Diseases*. London, 1900. P. 489.
 2. Manson, *The Filaria Sanguinis Hominis*. London, 1883.

TABLE III

No. OF FILARIAE PER DROP OF FINGER BLOOD

		A.M.										P.M.											
DATE		4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	
Case 1.	10 viii. 79	17	
	11 "	0	0	16	
	12 "	0	0	26	
	13 "	0	0	14	
	14 "	2	2	6	
	15 "	0	0	4	
	16 "	0	0	26	
17 "	2	0	12	
Case 2.	15 vii. 79	1	13
	10 viii. 79	1	0	25	
	11 "	2	0	
	12 "	1	3	
Case 3.	16 vi. 79	43	
	17 "	6	2	1	0	24	57	
	18 "	23	1	0	0	105	21	
	19 "	18	0	0	0	...	1	10	29	37	
	20 "	15	0	0	0	29	89	
	21 "	2	1	0	1	53	41	
	22 "	2	0	0	0	17	34	
	23 "	5	0	0	0	24	43	
	24 "	23	0	0	0	14	
	25 "	7	0	0	0	10	13	
	26 "	14	0	0	0	19	
	27 "	11	0	0	10	
	28 "	5	0	0	12	
	29 "	17	0	0	13	
	30 "	14	0	0	0	12	35	
	1 vii. 79	33	1	
Case 4.	20 viii. 79	1	0	5	
	21 "	0	0	0	
	22 "	0	0	1	
	23 "	0	0	0	
	24 "	0	0	2	
	25 "	0	0	12	
	26 "	0	0	2	
	27 "	1	0	0	

Table IV, showing experimental inversion of filarial periodicity constructed from MANSON'S Chart.—No. of filariae in preparation under 1 x 1½ inch cover glass.

DATE				A.M.						P.M.						
				2	4	6	8	10	12	2	4	6	8	10	12	
9. xii. 79												0		58		
10 „						63		0	0			0		62		
11 „						96		6	0		0	10		30		
12 „						155		8	0		0	8		88		
13 „						70		6	0		0	8		26		
14 „						48		7	0			0		38	115	
15* „						185		12	6			0		8	12	
16* „						52		46	8			6		8	28	
17* „						38	8		70	14			6		8	6
18* „						8	48		38	60			5		5	8
19* „						5	74		76	50		88	5		4	6
20* „						8	38		62	52			10		2	8
21* „						15	42		36	34			11		10	11
22* „						11	28		60	46			10		6	6
23* „						15	54		48	95			24		8	4
24* „						12	21		68	86			18		8	8
25 „						9	15									

We have had to construct this table from a chart in which the number of filariae were recorded by dots placed between horizontal lines, each representing ten filariae, and thus the numbers may not be exactly correct (within one to five units), owing to the difficulty of gauging the number represented by a dot placed between such lines.

On days marked thus * the sleeping hours were from five a.m. to five p.m. On other days from six p.m. to six a.m. On December 14 the patient was not allowed to sleep. The experiment had been previously made by MACKENZIE¹ with similar results.

1. Mackenzie, *Trans. Path. Soc. of London*, vol. xxxiii, p. 400.

The following tables illustrating the periodicity of *F. nocturna* have been constructed from our own notes of a number of cases among West African natives. Three specimens were made from each case every three hours. Sufficient blood was taken, to form as nearly as possible a complete film under a cover glass three-quarter inch square, and the specimens were examined in the fresh condition. Throughout the following tables the maximum number of filariae in three slides is indicated by a larger type of figure.

TABLE V

NAME	DATE	NUMBER OF FILARIAE IN THREE SLIDES AT							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Oparobo ...	11. vii. 00 ...	20	0	0	0	0	10	48	26
2. Deafman ...	12. vii. 00 ...	0	2	0	0	0	0	1	3
3. James ...	12. vii. 00 ...	7	0	1	0	0	0	11	9
4. Abraham ...	27. viii. 00 ...	0	1	0	1	0	0	4	2
5. Onye mensoh ...	27. viii. 00 ...	18	2	0	0	0	25	45	56
6. Sumanu ...	27. viii. 00 ...	7	0	0	0	0	17	21	9
7. Osadebe ...	27. viii. 00 ...	28	2	1	0	0	35	34	50
8. Eyamah ...	27. viii. 00 ...	2	0	0	0	0	3	7	19

As to the periodicity of *F. diurna*, MANSON¹ says simply that the parasites come into the blood during the day and disappear from it during the night; and, the periodicity observed by the parasite was thoroughly made out by prolonged observation in one of the cases. Actual records we have not been able to find.

From our own collection of records of cases we have constructed the following table illustrating the periodicity of *F. diurna*: the figures represent the number of embryos in three specimens of blood under a three-quarter inch square cover glass.

1. Manson, *Tropical Diseases*, London, 1900. P. 532.

TABLE VI

NAME	DATE	NO. OF FILARIAE IN THREE SPECIMENS AT							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Robert ...	12. vii. 00 ...	0	8	84	58	66	47	0	0
2. Adeyemi ...	27. viii. 00 ...	0	3	8	48	7	3	0	0
3. Obudu ...	27. viii. 00 ...	0	10	21	32	...	8	1	0
4. Garuba ...	27. viii. 00 ...	0	1	27	52	38	9	0	0
5. Apanituen ...	20. vii. 00 ...	1	3	7	8	3	0	0	0

It has not been easy to pick out from our records a fair number of cases either of *F. nocturna* or *diurna* which may be said to be absolutely typical ; thus only eight cases of *F. nocturna* and only five of *diurna* could be found. We propose to call a case typical when the maximum number of embryos are present in the blood at mid-day or midnight as the case may be, or about those hours and when twelve hours later they are absent from peripheral blood.

The majority of the cases which we encountered on the West African coast were then atypical, in that, embryos were never absent from peripheral blood, or the maximum did not occur at mid-day and midnight or thereabouts according to the species. Among the former cases there were many shewing decided periodicity and among the latter, the hour at which the maximum number was present, varied considerably. In some cases two maxima during the twenty-four hours were indicated. Table VII shews a few cases in which though a decided periodicity is to be noted, embryos are never absent from peripheral blood.

TABLE VII

NAME	DATE	NUMBER OF EMBRYOS IN THREE SPECIMENS AT							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Davis ...	12. vii. 00 ...	12	61	425	478	197	252	12	9
2. Ajaca ...	27. viii. 00 ...	9	20	10	5	...	3	35	18
3. Arrigwe ...	3. vii. 00 ...	7	6	16	58	58	45	15	4
4. Ijululockia ...	20. vii. 00 ...	15	18	56	61	25	1	1	3

Table VIII gives a number of cases in which the maximum number did not occur at mid-day nor midnight.

TABLE VIII

NAME	DATE	NUMBER OF EMBRYOS IN THREE SPECIMENS AT :							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
Etta ...	3. vii. 00	12	3	8	20	46	25	21	21
Jumbo ...	3. vii. 00	0	1	2	4	18	4	0	0
Efon ...	12. vi. 00	0	11	7	5	16	6	0	0
Greenslade ...	12. vii. 00	0	1	28	54	76	81	15	0
Glasgow ...	12. vii. 00	4	35	...	9	20	49	3	0
Joe ...	12. vii. 00	3	0	0	0	0	4	12	6
Oparobo ...	12. vi. 00	20	0	0	0	0	10	48	26
Kelba ...	12. vii. 00	2	1	0	3	5	8	15	7
James ...	12. vii. 00	7	0	1	0	0	0	11	9
Abraham ...	27. viii. 00	0	1	0	1	0	0	4	2
Sumana ...	27. viii. 00	7	0	0	0	0	17	21	9
Ajaca ...	27. viii. 00	9	20	10	5	...	3	35	18
Emordi... ..	27. viii. 00	54	5	0	3	3	15	22	44
Arrigwe ...	12. vi. 00	14	25	5	27	8	18	5	8
Mark ...	12. vi. 00	2	66	28	22	...	18	2	1
Okohorsfall ...	20. vii. 00	42	43	54	26	25	29	5	9
Deauma ...	20. vii. 00	4	60	130	68	47	1	1	0
Robert ...	12. vii. 00	0	8	84	58	66	47	0	0
Etim ...	12. vi. 00	3	6	16	6	8	0	0	0

THORPE¹ examined a number of natives of the Friendly Islands; but his results, as recorded in the article referred to, do not permit very definite conclusions. We have however reproduced them in the following tables copied from his article.

1. Thorpe, *British Medical Journal*, 1896, vol. ii, p. 922

TABLE IX

	NATIVES OF TONGATABU			NATIVES OF NORMUKA			NATIVES OF LIFUKA			NATIVES OF VAVAU			TOTALS		
	No. exam.	No. infect.	Per- centage	No. exam.	No. infect.	Per- centage	No. exam.	No. infect.	Per- centage	No. exam.	No. infect.	Per- centage	No. exam.	No. infect.	Per- centage
MALES EXAMINED :															
Day and night ...	7	3	...	25	12	...	23	12	55	27	49
Day only...	3	0	...	3	0	...	2	1	8	1	...
Night only ...	31	11	...	9	3	...	6	5	...	14	4	...	60	23	...
	41	14	34.1	37	15	40.5	31	18	57	14	4	28.6	123	51	41.47
FEMALES EXAMINED :															
Day and night ...	4	3	...	26	7	...	11	3	41	13	31.7
Day only...	3	0	...	6	0	9	0	...
Night only ...	17	2	...	11	1	...	7	2	...	6	0	...	41	5	...
	24	5	20.8	43	8	18.6	18	5	28	6	0	...	91	18	19.9
Totals for whole population ...	65	19	29.23	80	23	28.75	49	23	47	20	4	20	214	69	32.24

TABLE X

NAME	A.M.			P.M.				
	9 $\frac{1}{2}$	10	12	2 $\frac{1}{2}$	5 $\frac{1}{2}$	6 $\frac{1}{2}$	8 $\frac{1}{2}$	10
Tubon	21	9	16	17	...	30	...
Saen ...	22	...	27	16
Kesaia	80	56

The numbers represent the number of embryos in a drop of blood under a seven-eighths inch circular cover glass.

Referring to the day and night examinations, THORPE says that no periodicity was observed: that the embryos resembled *F. nocturna*; they had a sheath, and exhibited the characteristic preputial collar and V-spots. He gives a number of measurements which correspond to those of *F. nocturna*, except that the worm appears to be a little smaller than that of China and India. In ninety-six cases examined, all

except two had an equal number of embryos in the blood both day and night : of the two exceptions, one showed a single parasite at night, none in the daytime ; the other a single parasite at the day examination, none at night.

In spite of the small number of examinations and of their incompleteness, it is certainly evident from the above figures that the parasite does not agree in any way in its occurrence in the peripheral blood with either *F. diurna* or *F. nocturna*. It must however be noted that THORPE describes the Friendly Islands as 'a hot-bed of elephantiasis.' This point will be referred to later.

The following tables illustrate how the occurrence of embryos in the blood varies from day to day and week to week in the same cases. It must be here remarked, that the habits of the men whose blood was frequently examined for the purpose of the construction of these tables, were marked by extreme regularity. They were government prisoners, kept in the government prison at Bonny. The men rose at five o'clock, were fed at eleven o'clock mid-day, and were locked up in their cells about eight o'clock ; from five till eleven and from twelve till six they were at work.

In every case three drops of blood were examined under a three-quarter inch square cover glass.

TABLE XI

NAME	DATE	NUMBER OF FILARIAE IN THREE BLOOD SPECIMENS AT							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Arrigwe	12. vi. 00	14	25	5	27	8	18	5	8
	3. vii. 00	7	6	16	58	58	45	15	4
	7 "	18
	9 "	38
	10 "	43
	11 "	89
	12 "	13
	13 "	32
	14 "	53
	12. vi. 00	46	50	27	26	24	6	5	17
	3. vii. 00	12	3	8	20	46	25	21	21
	7 "	17
	9 "	4
	10 "	10
2. Etta...	11 "	17
	12 "	14
	13 "	9
	14 "	9
	12. vi. 00	0	11	7	5	16	6	0	0
	3. vii. 00	0	4	16	15	10	1	0	0
	9 "	9
	10 "	13
	11 "	36
	12 "	52
	13 "	18
	14 "	56
	12. vi. 00	1	4	8	1	4	0	0	0
	3. vii. 00	0	4	16	15	10	1	0	0
3. Eñon	9 "	10
	10 "	0
	11 "	2
	12 "	2
	13 "	7
	14 "	6
	12. vi. 00	3	6	16	6	8	0	0	0
	3. vii. 00	0	0	17	9	8	5	0	0
	9 "	26
	10 "	6
	11 "	23
	12 "	14
	13 "	6
	14 "	10
4. Jumbo	12. vi. 00	1	4	8	1	4	0	0	0
	3. vii. 00	0	4	16	15	10	1	0	0
	9 "	10
	10 "	0
	11 "	2
	12 "	2
	13 "	7
	14 "	6
	12. vi. 00	3	6	16	6	8	0	0	0
	3. vii. 00	0	0	17	9	8	5	0	0
	9 "	26
	10 "	6
	11 "	23
	12 "	14
	13 "	6
5. Etim	14 "	10

In this table the point to be noticed is that a considerable amount of variety occurs in the way in which embryos present themselves in the peripheral circulation in those cases in which the type (*F. nocturna* or *F. diurna*) is not strictly adhered to.

For instance, in Cases 3, 4, and 5, the numbers at each examination are as near as would be expected; but in Cases 1 and 2 the variations from day to day are considerable.

In the above tables, III to XI inclusive, a further feature is to be observed, namely, the variety in the severity of the filarial infection: thus, taking the numbers of embryos at the period when they reach a maximum in peripheral blood, it is seen that they are included between 3 and 480 in three specimens, or 1 and 160 per specimen of blood. It surely follows, then, that in some natives even when they are at their maximum number, in the peripheral blood, they may still be too few, *in toto*, to be observed in a single preparation of blood. Consequently, many more natives must be habitats for filariae than is supposed from the observation of peripheral blood in the usual way. When treating of *F. perstans* (see Table XII, Case 4), it will be seen that in some sixty preparations of the blood of one case, one filaria only was observed (possibly the infection with *F. perstans*, or the maturation of the parasite, may have occurred during the month under which the case was under observation). MANSON's¹ figures shew the same features in the cases of undoubted *F. nocturna* infection; but few of these figures, however, give the number of embryos per drop of blood when the largest number would have been present in peripheral blood, namely, twelve midnight: most of the specimens were made not later than ten p.m.: the figures range between 1 and 105. These facts must be taken to give some indication of the severity of the infection, of the number of adult females in the organism: since the results of observations extended over a long period—a month or more—shew no decided periods of increased fertility. But it surely must not be inferred from the relative numbers of embryos in the two extreme cases that the number of adult females in one case is a hundred or more times as many as in another, although it is difficult, at the present stage of our knowledge, to understand why such an inference should not be drawn. Referring to this subject, MANSON² is reported to have said—‘If anyone is foolhardy enough to submit to be bitten by filariated mosquitoes, and if subsequently no young filariae be found in the blood, it must not be concluded from this that a mosquito bite is not the medium of infection. My belief is that before embryos can be found in the blood by ordinary microscopic observation large numbers of parent filariae must be present in the lymphatics. In many cases we know that hundreds of parent filariae are present. Thus in one case only two or three embryo filariae are found in each drop of blood; in other instances as many as 600 or more are found in a drop implying the presence of 300 times as many parental worms.’ Although as above stated we do not at present understand why such an inference cannot be deduced, it is evidently not justifiable to make such an inference, judging from the number of infected inhabitants and the extent of their infection. We have not been

1. Manson, *The Filaria Sanguinis hominis*. London, 1883.

2. Manson, *Brit. Med. Journal*, Sept. 1. 1900. P. 536

able to find any record of a case or cases in which the embryos were regularly counted for a period shortly before death and in which, *post-mortem*, adults were found.

In the examination for malarial parasites¹ of blood specimens from a large number of native children of all ages up to about 18 years, we encountered a single filarial embryo only, in one case (specimens taken during the day were examined only)—aged 11 years, out of 390 cases. In view of the number of adults infected with *F. diurna* in the same districts, this is remarkable and further tends to support the idea that, the extent of infection increases during the period of childhood, until, when adult age is reached, there are a sufficient number of mature female filariae in the body to give an observable number of embryos in peripheral blood during the usual examination for microscopical purposes.

Under any other circumstances, it seems to us, there would be no chance of an escape from the continued and renewed infection of every individual. In a certain district, were such a number of embryos observable in the blood of every child—or even of a similar percentage of children, as is presented by the adults, every mosquito of the species capable of acting as intermediary hosts would become infected and in consequence every man, woman and child in that district would become infected to such an extent as to exhibit embryos in the peripheral blood. It thus seems that in this way nature has placed a limit to the prevalence of this infection.

Filaria perstans

But little need be said of the periodicity of this worm, which persists in the peripheral blood throughout the whole of the day. The following table illustrates the phenomenon.

1. *Report of the Liverpool Malaria Expedition to Nigeria*, Liverpool, 1901, part i, p. 11 *et seq.*

TABLE XII

NAME	DATE		A.M.				P.M.			
			3	6	9	12	3	6	9	12
1. Ekpeyon ...	12. vi. 00	...	0	7	1	1	0	2	4	3
	3. vii. 00	...	2	2	5	3	5	5	1	4
	9 "	6
	10 "	3
	11 "	10
	12 "	3
	13 "	2
	14 "	2
2. Etim ...	12. vi. 00	...	1	1	0	0	0	0	4	1
	3. vii. 00	...	2	2	1	1	0	4	4	1
	9 "	1
	10 "	2
	11 "	2
	12 "	1
	13 "	0
	14 "	10
3. Joe ...	12. vi. 00	...	3	0	2	2	0	4	0	1
4. Efon ...	12 "	...	0	0	0	0	0	0	0	0
	3. vii. 00	...	0	0	0	0	0	0	0	0
	10 "	0
	14 "	1
5. Ijuluockia ...	19 "	...	0	1	9	3	7	7	3	2
6. Demai ...	19 "	...	1	0	2	2	0	0	1	0
7. Malam ...	27. viii. 00	...	0	2	2	7	2	1	4	2
8. Obudu ...	27 "	...	3	2	0	0	...	0	1	2
9. Sumanu ...	27 "	...	1	1	0	0	0	0	4	1

The only striking feature which this table presents is the smallness of the number of *F. perstans* embryos ; the greatest number we ever observed, was thirteen in three specimens under three-quarter inch square cover glasses ; that is, approximately 2 to 3 c.mm. of blood.

THE INTERMEDIARY HOST OF *FILARIA NOCTURNA* : ITS DEVELOPMENT.

The phenomenon of the periodicity of *F. nocturna* led MANSON to induce the further development of the parasite in a blood-sucking insect of nocturnal habits. In 1878 MANSON demonstrated developmental changes in the embryos after ingestion by the mosquito, since when the whole of the life history in the intermediary host has been observed by BANCROFT, JAMES, SONSINO, LOW and ourselves in *Culex pipiens*, *C. ciliaris*, *C. fatigans*, *Anopheles costalis*, *Anopheles rossii*, BANCROFT has shewn that *C. notoscriptus* (SKUSE), *C. amuli rostris* (SKUSE), *C. hispidosus* (SKUSE) *C. vigilax* (SKUSE), *C. nigrothorax* (MACQUART), *C. procax* (SKUSE) and *Anopheles musivus* (SKUSE) do not serve as intermediary hosts.

MANSON¹ gives the details of the various stages of the metamorphosis of *F. nocturna* embryos in *C. pipiens*.

First Stage. Transverse striation becomes well marked as if from a general longitudinally shrinking of the embryo ; oral pouting vigorous. In about one hour the embryo casts its sheath ; and then shows active locomotive movements. In from twelve to eighteen hours many have bored through the stomach wall of the mosquito and have reached the muscles of the thorax. Some die in the stomach. In the thorax, the striation disappears and movement ceases : the body becomes thicker and an illdefined cloudiness appears in the interior.

Second Stage. The body thickens, and there is a faint indication of a mouth ; this stage requires two to three days for completion.

Third Stage. The anus appears, and cells are seen in the body ; the mouth becomes open, and gradually four large fleshy lips are fashioned. The anus appears in front of the tail as a break or hole in the cuticle, from which granular matter exudes. The line of the cells, which are now visible in the previously apparently homogenous body, does not terminate at the anus but in advance of this, in some large prominent cells. The cells later become differentiated into an alimentary layer, and a tegumentary layer with a cavity between. The larva now measures $\frac{1}{800}$ to $\frac{1}{80}$ inch long (0.25 to 0.3 mm.) and $\frac{1}{850}$ to $\frac{1}{500}$ inch broad (0.048 to 0.45 mm.) There is considerable diversity in size and shape. The mouth is wide open ; the tail is large and sickle shaped, and the cells of the body usually dip into it. The alimentary canal runs from mouth to anus. Motion is entirely suspended.

Fourth Stage. Growth is rapid : length $\frac{1}{70}$ to $\frac{1}{50}$ inch (0.35 to 0.5 mm.) The body retracts from the tail, which becomes a mere integumental appendage.

1. Manson, *Transactions of the Linnean Soc.*, 1884. P. 367

Fifth Stage. When the body has attained its maximum thickness, lengthening and thinning begin at the head end. The mouth inclines to purse up. The anterior and posterior ends may elongate simultaneously; more generally the process occurs throughout the whole length of the body of the larva. When the mouth closes, as it does later, all or nearly all trace of viscera and all traces of cells vanish. About the seventh day the body assumes a fibrous and very transparent appearance. Before this stage there can be made out a fully moveable alimentary canal, pharynx and oesophagus. Slight movements commence at the neck of the animal and extend downwards. MANSON thinks that about this stage a general ecdysis occurs, and the sickle shaped tail is cast off: a new skin can be seen covering the tail end, inside the sickle. Large cells appear at the end of the tail and form three or four papillae which characterise the larva at the end of this and during the next stage.

The worm has now reached a length of $\frac{1}{16}$ inch in length (1.5 mm.), its breadth has decreased to about one-half. The anterior end tapers and is abruptly rounded off; the posterior end also tapers slightly from the anus backward and is covered by the papillae just mentioned.

Sixth Stage. Movements become more active. The mouth is pursed up into a cone with lips firmly approximated; minute horny papillae are present. . . . The worm measures $\frac{1}{16}$ by $\frac{1}{850}$ inch (1.5 mm. by 0.03 mm.)

Up to 1900, this was supposed to be the complete development of the filaria in the mosquito, and at this stage it was conjectured that, on the death of the mosquito on the surface of the water, the young filaria escaped from the insect and swam about until it was taken up by man in drinking water.

In 1900 Low¹ in sectioning a number of filariated mosquitoes discovered a worm in the proboscis. He thus describes the transformation into the seventh stage:—‘When the filariae have reached their highest stage of development in the thoracic muscles, they leave that tissue and travel forward in the direction of the head of the mosquito and pass into the loose cellular tissue which abounds in the prothorax near the salivary glands. Some struggle between the thorax and abdomen or within the abdomen itself. They then pass into the neck, enter the lower part of the head and coil themselves up in the loose connective tissue immediately below the cephalic ganglion and salivary sack. They pass into the proboscis by making an independent passage through the base of the labrum and pushing forward along the proboscis between the labrum and hypopharynx amongst the stilettes. Here they are found stretched along the length of the proboscis, head foremost. Two worms nearly always live together in the proboscis.’

JAMES² apparently was working at this subject at about the same time, and writes in an article, dated September, a description of the worms as seen in *Anopheles*

1. Low, *British Medical Journal*, 1900, June 16

2. James, *British Medical Journal*, 1900, vol. ii, Sept. 1, p. 535.

rossii on the seventeenth and eighteenth days of cultivation. 'The young filariae are found in the tissues of the thorax, in those of the head and neck, and in fewer numbers in those of the abdomen. The tissues of the head are examined by cutting through the neck. . . . By carefully dissecting with needles the tissues of the head, and separating the parts of the proboscis, two or three filariae will almost invariably be found in this situation, and I have lately on two occasions found a filaria lying stretched out lengthwise partly within the tissues of the labrum of the proboscis, the remainder of its body being curled up in the tissues of the head. Without dissecting up the tissues of the labrum these filariae could be plainly seen with a $\frac{1}{6}$ inch objective through its fairly transparent tissue indulging in sinuous undulatory movements, and a very little manipulation with the needles sufficed to free the filariae when their movements changed from the snake-like undulatory character to the vigorous purposeless lashing and twisting which are characteristic of the final stage of the metamorphosis of the parasites in the mosquito. In the diagram I have shown the appearance of the filaria as it lies partly within the labrum. . . . The young filariae in the final stage are from $\frac{1}{14}$ to $\frac{1}{16}$ of an inch in length, and $\frac{1}{800}$ inch in greatest breadth. It tapers towards the head and tail; the latter has three projections which can be spread out or drawn closely together in the animal's movements. The head end is rounded, and the mouth which is very extensile can be pushed out to form a little cone-like projection which sways from side to side, and is drawn in and pushed out as if searching for food. The filariae have an alimentary canal which at a somewhat earlier stage can be seen to be very freely moveable within the animal's body, and to be of varying shape in different parts of its course. Near the anus it is wide, and then narrows gradually to open at a short distance from the tail. On each side of the alimentary canal near the head, and again at a point about the middle of the body, the protoplasm is differentiated into other organs—probably reproductive.'

On August 4, 1900, as a result of some experiments which we had been carrying on as opportunities presented, during the time we were in Southern Nigeria, we were able to cable home that a living filaria had been found in the proboscis of *Anopheles costalis*. Previous to this several attempts had been made by us to cultivate both *F. nocturna* and *F. diurna* in mosquitoes of both genera, *Culex* and *Anopheles*, but without success. In this experiment with *F. nocturna* we were however successful. We had on several occasions previously noticed that large filariae were to be seen in the head of mosquitoes. The mosquitoes were fed on two occasions on blood containing embryos—July 18 and July 20, and in order to keep them alive for a period they had occasionally been fed on blood containing no embryos. On August 4 only five *Anopheles* of the batch remained, two of them being dead on the water. One of these on dissection proved negative. In the other, in the proboscis and near a trachea in the labium of that organ was a long thin filaria. In the thorax of this

mosquito a similar worm of the same size was found. A living mosquito was then taken, quickly killed by chloroform, and without any dissection whatever the thorax was pierced by a needle, and the finger nail placed on the tip of the proboscis. The parts were then gently drawn apart, the labium and palpi being thus separated from the stylets. A very active filaria was then seen to curl itself out from the neighbourhood of the trachea of the labium. This lived for about an hour in normal saline, coiling and uncoiling itself. The other two *Anopheles* died during the following night and on dissection proved to be negative.

In this stage, *the seventh*, the worm according to our specimen, is about 1.006 mm. long and 0.025 mm. broad. It tapers slightly to each end. At the anterior end, which is rounded off, the cuticle is thickened in places to form a few very small papillae disposed around the oral orifice, which is terminal. The posterior end, which is also rounded off, is provided with four papillae which are almost at right angles to the axis of the body of the worm. The position of the anal orifice cannot be definitely decided. The alimentary canal can be seen to run straight down the worm and shows no differentiation as far as we have been able to ascertain in oesophagus and intestine. Besides the alimentary tube in parts two other tubes can be seen which are for the most part straight, but at one or two points seem to twist round the intestine. Towards the head end at a distance of 0.14 mm. from the anterior end, there is an indication of the presence of an orifice towards which the reproductive tube is seen to bend.

FILARIAE IN ANOPHELES COSTALIS

According to our notes 281 *Anopheles* were examined for filariae—sixteen of these (5.7 per cent.) were found to contain the worms. The following are the details of the examinations :—

1. Two large filariae.
2. Eight to ten among thoracic muscles.
3. Several young larvae.
4. About ten large forms in thorax.
5. Several large forms among the thoracic muscles.
6. A single large filaria.
7. A small larva dissected out from the head.
8. Eight small larvae in thorax.
9. A few young forms in thorax.
10. Four young larvae.
11. Ten larvae found.
12. Fourteen larvae in thorax.
13. Several large larvae.
14. A single larva.
15. A single large larva found at the base of the proboscis.
16. Ten filariae in thorax.

The Anatomy of the Mouth parts of the Female *Anopheles Costalis*

The discovery of the final stage of the metamorphosis of the larval stage of *F. nocturna* in sections of the proboscis of mosquitoes, by Low,¹ in 1900; the observations of JAMES² as to the way in which the matured larvae tend to travel through the tissues of the head and neck into the proboscis; the work of GRASSI³ on *F. immitis* in the dog, and in *Anopheles claviger*, in which the larvae were found in the labium; and our own researches in West Africa on the life history of *F. nocturna* in the body of *Anopheles costalis*; make a knowledge of the minute anatomy of the proboscis of the mosquito requisite for an exact understanding of how the larva leaves the insect and is transmitted to man.

As far as we have been able to ascertain very little has been done by entomologists in this country in the investigation of the histology of the proboscis of the mosquito; it has chiefly been examined from a morphological point of view, as of importance in the classification of species.

METHODS OF INVESTIGATION EMPLOYED

1. Examination of the proboscis of living insects.

2. Dissection of specimens in the fresh condition; and the examination of the organ hardened in alcohol and cleared in oil of cloves, and mounted as a whole or in parts in Canada Balsam.

3. By sections. Mosquitoes were killed by chloroform vapour; hardened in absolute alcohol for one or two hours, and embedded in paraffin. Serial sections (6 to 10 μ thick) of the proboscis and head were cut in three directions. Thin paraffin sections of the proboscis are with difficulty fixed to a slide by the ordinary methods of laboratory practice; the chitinous skeleton tending to break away from the delicate tissues which it encloses, in the processes of the manipulation of paraffin sections, so that minute anatomical relations become disturbed and obscured.

By the use of a slight modification of OBREGIA'S⁴ method for fixing sections cut in paraffin, we were able to secure excellent results. A mixture of two parts of commercial liquid glucose and one part of a thick syrup of pure dextrin* is spread in a thin layer on to the glass slide by means of a glass rod. The serial paraffin sections as they are cut are laid directly on this layer on the slides, which are then placed in an incubator at about 40° c. for some hours, until the glucose mixture has dried hard. The paraffin is then removed by means of xylol and the slide with the sections is passed through absolute alcohol. A solution of photoxylin is then poured over the slide, so as to form a thin film over the sections; this layer of photoxylin is

1. Low, *British Medical Journal*, 1900. June 16.

2. James, *British Medical Journal*, 1900. Vol. ii, Sept. 1, p. 535.

3. Grassi, *British Medical Journal*, Nov. 3, 1900.

4. Obregia, *Neurologisches Centralblatt*, 1890.

Gulland, *Journal of Pathology*, Feb. 1893, p. 391.

* 16 oz. dextrin; 17½ oz. water; 15 grains thymol.

allowed to set until the edges of the film begin to crinkle. On placing the slide in water, the film comes away with the sections which are now ready for staining *in situ* in the film. Carbol-xylol must be used for clearing after dehydration.

Most of our observations were made on *Anopheles costalis*, but a few specimens of *Anopheles maculipennis* and of different species of *Culex* have also been examined by dissection.

EXTERNAL ANATOMY OF MOUTH PARTS

The term 'proboscis' is used to designate such of the mouth parts of *Diptera*, which taken together form their more or less flexible, shorter or longer, sucking apparatus. In the Culicidae the proboscis is a long slender organ arising from the lower projecting portion of the front of the head, beneath the clypeus or face. On its ventral surface it is continuous with the under surface of the head—the gulo-mental region. Its upper surface is sharply marked off from the clypeus by a deep groove. In a transverse section of the head at the base of the proboscis, (plate XVI, fig. 2) the latter appears to arise from a U-shaped mass under the clypeus, the upper parts of the arms of the U representing the genae or cheeks—narrow areas of the head situated in front of the eyes. The proboscis measures as a rule about three or four times the length of the head; in *Anopheles costalis*, 2 mm.

PARTS CONSTITUTING THE PROBOSCIS

The proboscis consists of the upper lip—the labrum; the epipharynx; these two being firmly united together; the hypopharynx or tongue; two mandibles and two maxillae, which are commonly known as the stylets or setae, consisting almost entirely of transparent chitin, and used to pierce the skin; and the labium, or lower lip, the largest and fleshy part of the proboscis, in a groove on the upper surface of which the other parts are ensheathed when in repose. On either side and above the labium are the two maxillary palps, rod-like organs, covered with hairs and scales, and which, in *Anopheles*, lie above and parallel to the other mouth parts, and extend almost to the tip of the proboscis.

The general arrangement of the mouth parts to one another is seen in plate XV, fig. 3, a transverse section about the middle of the proboscis.

The epipharynx. The central tube through which the blood is sucked is formed by the epipharynx, which is morphologically the continuation of the upper and lateral chitinous walls of the pharynx. This tube is tunnel-shaped, being flattened on its under surface; its distal open end is oval, and looks ventrally—a fact first pointed out by SWAMMERDAMM¹ in 1668. The wall of the epipharynx on the ventral surface becomes exceedingly thin, and fails to meet in the middle line, so that a slit is formed running the whole length of the epipharynx. The tip of the epipharynx ends

1. Swammerdam, *Buch der Natur*. Leipzig, 1752.

in a sharp point, and presents the appearance of the point of a pen, having a central split and small eye. It is composed of two conical pieces, the bases of which blend with the upper rounded wall of the tube—and a slight thickening at the junction of the two pieces in the middle line gives rise to the appearance of the slit of the pen. On each side of the epipharynx, at its base, and intimately blend with it, is a stout rod of chitin having a core of large nucleated cells; this rod is the continuation of the lateral horizontal plate of chitin which at the base of the epipharynx affords attachment to the epipharyngeal muscles. The outer edge of it turns gradually upwards and inwards, and, fusing with the lateral convex surface of the epipharynx, forms the lateral supporting rod of chitin described. In transverse sections the core of nucleated cells in its interior is seen to be continued down the whole length of the epipharynx and at its distal end, the core turns upwards and towards the middle line; the epipharynx thus forming the extreme tip. The labrum, which is intimately blended with the epipharynx superiorly, thus takes no part in the formation of the extremity, stopping short before the nib-like tip is reached.

The interior of the epipharynx measures at its base, dorso-ventrally $19.8\ \mu$, from side to side $26\ \mu$; at the middle of the proboscis $16.5\ \mu$ dorso-ventrally, $18.1\ \mu$ across; and at the middle of the labellae $13.5\ \mu$ vertically by $13.2\ \mu$ across.

The **labrum** or upper lip is a delicate chitinous process situated immediately above the epipharynx and intimately connected with it, in fact it can be only partially separated from it by such reagents as caustic potash. For this reason DIMMOCK¹ described them as one piece—the labrum-epipharynx. The labrum arises at the base of the clypeus and runs along the upper surface of the epipharynx. In a transverse section near the base of the proboscis (plate XVI, fig. 1), it is seen that the labrum is composed of a curved lamella of chitin with its convexity approximated to the convexity of the upper surface of the epipharynx. The sides of the superimposed furrow thus formed, lower down the proboscis, suddenly become thinned, and, turning outwards and downwards are thrown into folds of very delicate chitin which unite below with the outer edges of the lateral rods of chitin of the epipharynx (plate XVI, fig. 1), the space thus closed in is occupied by loose cellular very delicate connective tissue. Towards its distal end, the furrow of the labrum becomes shallower and opens out, and the labrum itself becomes more intimately fused with the epipharynx. In sections (plate XV, fig. 1) near the tip of the proboscis the labrum-epipharynx is seen as a more or less triangular-shaped piece made up of three parts; two lateral pieces of chitin, in the centre of each of which is a deeply stained nucleus (chitin-cell); and a superimposed crescentic central upper piece united with the lateral portions by a very delicate band of tissue; this represents the tip of the labrum, which, as has been already described, stops short of the end of the epipharynx.

1. Dimmock, *The anatomy of the mouth parts and of the sucking apparatus of some Diptera*. Boston, 1881. P. 13.

At the proximal end the chitinous lamella of the labrum ends within the clypeus, projecting upwards for a considerable distance as a flattened rod-shaped piece, which affords attachment to fan-shaped muscles, arising from the roof of the clypeus.

The hypopharynx. SAVIGNY (1816); lingua, WESTWOOD; ligula, KIRBY and SPENCE (1828); or tongue, is formed by a prolongation of the chitinous lower wall of the pharynx. It is a thin, flattened lamella of chitin, closely applied to the under surface of the labrum-epipharynx. Its lateral edges are turned upwards slightly, and upon these rest the inner edges of the mandibles and the convex basal borders of the epipharynx. The tip of the hypopharynx is simple and lanceolate. In the centre of the hypopharynx the chitin is thickened and deeply hollowed out on its upper surface, to form an almost completely closed gutter running down the whole length of the organ, and approximated to the slit on the under surface of the epipharynx. The hypopharynx consists of an upper thick flattened plate of chitin, hollowed at its centre to form the gutter, and a lower thin plate; the intermediate space being filled with delicate connective tissue, and is lined with chitin forming cells: well seen in a section at the base of the proboscis (plate XVI, fig. 1). Throughout the distal two-thirds the two plates are fused together, the space remaining as a core of cells, imbedded in the chitin on each side of the salivary gutter. This gutter commences as a V-shaped opening (plate XIX, fig. 1) at the point of origin of the hypopharynx. Connected with this aperture is the salivary receptacle (plate XVIII, fig. 1 *s.r.*), a hollow, cone-shaped organ, lying applied to the ventral wall of the pharynx. The base of the cone points backward and slightly upwards; the apex, after a slight curve upwards, opens on to the salivary gutter at the V-shaped slit. The sides of the receptacle are of thick opaque chitin, except on its dorsal surface, which is somewhat flattened and composed of thin membranous transparent chitin. The lateral walls of the receptacle are strengthened by chitinous bands from the lateral portions of the clypeus. The base of the receptacle is distinctly membranous in character, very faintly staining with haematein: a little below its centre the common duct of the salivary glands is inserted. Above and around the insertion of the duct are attached the fine tendons of two muscles, one from each side (plate XVIII, fig. 1 *f.m.* and XIX, fig. 1 *r.m.*). These muscles arise together from the ventral surface of the lower chitinous plate of the pharynx, but more especially from a chitinous ridge on each side, which is concave anteriorly and also from above down, and projects from its under surface near the junction of the first and second portions of the pharynx (plate XVIII, fig. 1). The mechanism of the receptacle is probably as follows:—When the muscles contract, dilatation of the cavity of the receptacle is produced by pulling of the membranous base outwards; saliva then flows in and fills the cavity. On relaxation of the muscles, the membrane springs back into its original position; thus expelling the saliva down the channel of the hypopharynx.

The mandibles, two in number, are extremely delicate, transparent scroll-like rods of chitin, applied, one on each side of the base and sides of the epipharynx,

they are concave on their inner and convex on their outer surfaces, and are of uniform thickness for the greater part of their length, but for a short distance above their sharply-pointed tips they broaden, become more lance shaped and are twisted once upon themselves. In a transverse section at this level, they present several concavities into which the sides of the labrum-epipharynx, hypopharynx and maxillae fit (plate XV, fig. 1). Near their termination on the outer convex surfaces, lying along the upper edge is a row of very fine sharply pointed teeth varying in number, the sharp points projecting downwards. DIMMOCK¹ does not describe these teeth-like processes as occurring in the three species of *Culex* on which his observations were made. With regard to the origin of the mandibles DIMMOCK² says 'at the base of the proboscis they appear to have no muscular attachment but to lie embedded in the connective tissue beneath the pharynx and above the maxillae.'

In *Anopheles costalis*, plate XVI, fig. 2 shews their close relation to the inner surface of the base of the maxillary palpi, as a straight piece of chitin enclosing delicate cellular tissue. In sections further back they are difficult to trace but appear to come into relation with a downward projecting plate of chitin about the level of the anterior edge of the gena. They would thus appear to arise from chitin in the close neighbourhood of the groove between the clypeus and the gena. By tearing away the parts of the proboscis by traction at the tip with the finger nail, the mandibles come away with the maxillae attached to the maxillary palpi. To the base of each mandible a muscle is attached by a fine tendon; the muscle arises from the ventral surface of that part of the chitinous exoskeleton of the head, which is folded inwards beneath the eyes; the fibres are directed forward and slightly downwards.

The maxillae are two stouter lancet-shaped processes of chitin, one on each side; concave on their inner surface and fitting beneath the sides of the mandibles and the hypopharynx. On the upper and inner surface a slight distance from its inner edge runs a stout ridge of chitin from which the thinner portion of the maxilla curves upwards and outwards. The stout ridge is continued to the distal end of the maxilla, forming the sharp point. Some little distance from the point of the maxilla the thinner portion begins suddenly to shade off like the sharp edge of a penknife; this surface bears on its ventral side near the outer edge fifteen to twenty low conical chitinous papillae. DIMMOCK³ refers to them as being on the dorsal surface in *Culex*, and says 'they are true papillae, not points of a serrate edge.' The thinner portion of the shaft of the maxilla is marked with alternate light and dark bands at right angles to its longitudinal chitinous rod; this is due to the fine corrugation of its surface pointed out by DIMMOCK.⁴ This appearance is not

1. Dimmock, *The Anatomy of the Mouth parts, etc., of some Diptera*. Boston, 1881.

2. Dimmock, *Loco-cit.*, p. 16.

3. Dimmock, *Loco cit.*, p. 17.

4. Dimmock, *Loco cit.*, p. 16.

present on that portion of the maxilla from which the papillae arise. In a transverse section of the maxillae near their tips they present a peculiar jaw-like shape (plate XV, fig. 1). The maxillae appear to arise from the under surface of the maxillary palpi between them and the upper outer surface of the labium; in transverse sections they appear in this region as two lateral sickle-shaped stout masses of chitin, situated on each side of the commencement of the hypopharynx (plate XVI, fig. 2). Each maxilla is continuous with a thick rod of chitin, which extends almost the whole length of the head, ending in a long upper and a lower shorter stumpy rounded process in the basal part of the occipital region. To these intercranial chitinous rods, which appear to lie free in the cellular tissue at the base of the head, powerful muscles connected with the movements of the proboscis are attached (plate XIX, fig. 1; XVII, fig. 1; and XVIII, fig. 1). These processes have been variously termed; by LOWNE,¹ 'apodemes'; by MACLOSKIE,² the 'great tendons' of the mandibles. GERSTFELDT³ regarded them as the basal portions or 'cardines' of the hypopharynx. No mention is made of them by PACKARD⁴ in his description of the insects' mouth parts. SMITH⁵ regards these basal processes found in various genera of diptera *Bombylius*, *Antbrax*, *Eristalis*, *Musca*, etc., as basal prolongations of the palpifers (the mandibles of other authors), and states they may perhaps represent the 'stipides' as well—which he has not as yet identified in the dipterous mouth parts. It would seem from DIMMOCK'S⁶ account of the anatomy of *Culex* that these chitinous rods do not extend so far back into the head in this genus; he says 'their continuations (of the maxilla) in the head are two delicate chitinous supports, each of which ends in a strong muscle; this muscle—the retractor maxillae—passes backwards and downwards through the head beneath the infra-oesophageal ganglion, and has its origin in the posterior basal part of the head.' That they are the supports from which the maxillae arise can be well seen in serial sections.

The muscles in connection with the chitinous intra-cranial processes of the maxillae are:—

1. Muscles fixing them to the cranial exoskeleton. Each has a large muscle which arises from the lower occipital region of the cranium and is attached to the outer side of the process for the greater part of its extent: the fibres of this muscle run horizontally; muscle fibres, directed upwards, also run in connection with the terminal bifid extremity and that portion of the exoskeleton of the head, which is folded beneath the eyes (plate XIX, fig. 1 *z.m.*).
2. A spindle-shaped belly of muscle arises from the ventral surfaces of the processes to be inserted into the base of the labium (plate XVII, fig. 1; XIX, fig. 1 *l.m'*).

1. Lowne, *The Anatomy and Physiology of the Blow-fly*. London, 1870.

2. Macloskie, *The Proboscis of the House-fly*. American Naturalist, 1880, vol. xiv, p. 153.

3. Gerstfeldt, *Ueber die Mundtheile der saugenden Insecten*. Dorpat, 1853.

4. Packard, *Text-book of Entomology*. New York, 1898.

5. Smith, *Trans. American Entom. Soc.*, vol. 17, 1890, p. 338.

6. Dimmock, *The Anatomy of the Mouth parts, etc.* Boston, 1881. P. 16.

3. Muscle fibres arise from the superior surfaces of these processes, from a short length at their distal ends, and are directed upwards and forwards to be inserted into the upper surface of the first joint of the maxillary palpi.

Though the stylets appear to be wholly of chitinous structure yet in transverse section at their point of origin (plate XVI, fig. 1) it is seen that they really consist of a central prolongation of the delicate tissue lining the head, encased in a thick chitinous envelope under which is a row of flattened cells with large deeply staining nuclei ; these cells which secrete chitin can be traced almost to the tips of the labrum-epipharynx and hypopharynx. In the maxillae and mandibles traces of these chitin cells are seen in sections near the tips as a central staining core. (Plate XV, fig. 1 and 2). It is to be remarked how the shape of the stylets serve to bind them together, the convexity of the one above fitting into the concavity of the one below ; thus forming a solid chitinous awl with which the skin is pierced. A section at the tip of the proboscis illustrates the fitting of the stylets with one another (plate XV, fig. 1). In fact if the tip of the proboscis be cut off a little above the labellae, the stylets fall out of their labial sheath as one piece, nor do they separate unless pressure be applied. A good view is obtained in this way of the saw-like edges of the mandibles and maxillae, the latter being below and to the outside of the former.

The maxillary palpi. In *Anopheles*, the maxillary palpi are two long segmented rounded processes, thickly covered with hair and scales, lying in the resting condition, one on either side, on the upper surface of the labium and its enclosed stylets ; their tips are rounded off and end a little short of the tip of the proboscis. They are attached to the side of the head below on either side of the clypeus, their under surfaces here being in close relation to the maxillae (plate XVI, fig. 2). The basal joint is bulged on its upper surface ; on its under surface the chitin is thickened to form a ridge, which is in close relation at its proximal end to the chitinous prolongation of the maxillae. This joint contains muscle fibres arising from the maxillary prolongation ('great tendon') near its union with the maxilla, which are directed obliquely upwards and forwards to be inserted into the bulged upper surface.

Each palpi contain delicate connective tissue containing large cells: a nerve, comparatively large, arising from the lateral surface of the infra-oesophageal ganglion, and numerous very small tracheae. Muscle fibres are only present in the basal joint. The study of the maxillary palpi with regard to shape, size and surface markings is of great importance in the classification of species.

The labium or lower lip is the largest of the mouth parts and acts as a sheath for the stylets. It commences as a free piece in the same plane as the other mouth parts and is a continuation of the lower anterior part of the head below the pharynx. On its under convex surface it is marked off from the ventral surface of the head by

a slight groove which continues upwards on either side for a short distance, becoming deeper. Its convex ventral and lateral chitinous surfaces are thickly covered with hairs and scales, the chitin bearing them having irregular annular markings. Its upper surface is of smooth chitin, upon which the stylets rest. The labium tapers slightly from base to apex: at its commencement it is broad from side to side, its internal measurement from above down being $45.6\ \mu$; from side to side $65.2\ \mu$; its smallest depth is $22.8\ \mu$ and width $42.4\ \mu$. Its upper smooth surface is here flattened and on it rest centrally the hypopharynx, on either side the two maxillae (plate XVI, fig. 2). A little way from its origin, the labium becomes roughly round in shape owing to the edges of the upper surface turning upwards and inwards over the stylets forming a large oval channel in which they lie (plate XV, fig. 3); these edges are extremely fine and do not meet in the middle line, so that a space of uniform width is left running along the dorsal surface of the labium to its extreme tip. At about $0.16\ \text{mm}$. from the extremity of the proboscis the labium proper ends abruptly, while its upper concave surface is continued on to the tip of the proboscis, gradually tapering to a blunt point covered with fine hairs. This tip of the labium is easily broken off in dissections of the proboscis.

At the abrupt ending of the main portion of the body of the labium, which in transverse section is somewhat oval from side to side, are attached by true joints two lobiform appendages—the labellae—which enclose between their inner surfaces the tips of the stylets and the true tip of the labium. Crescentic at their bases, the labellae gradually taper to form the tip of the proboscis.

Running longitudinally on each side of the labium, and projecting into its substance from the inner surface of the chitinous exoskeleton, is a thick, very opaque chitinous ridge. These ridges commence at the base of the labium, and end abruptly a little distance behind the point of attachment of the labellae (plate XIX, fig. 3); from their inferior surfaces for about $34.3\ \mu$ from their distal extremities, and extending obliquely downwards and upwards, the chitin of the convex under surface of the labium proper becomes greatly thickened, forming two ventral plates, which in the mid-ventral line curve upwards and outwards, scrollwise, into the substance of the labium, ending in a short thick rod, near the centre of each lateral half of the labium (plate XIX, fig. 3 r). They present four borders: a proximal convex border continuous with the general exoskeleton of the labium; an outer border limited by the lateral longitudinal ridges of the labium; an inner, ending abruptly in its substance as a thick ridge of chitin; and a lower distal border, convex, curving from within, outwards, and upwards towards the distal end of the lateral ridge of the labium: upon the thickened inner extremity of this surface, which is hollowed out for its reception, the labella articulates. The labellae being removed, a view of the termination of the labium seen in section (plate XV, fig. 2) presents the following regions: on either side a pear or kidney-shaped area, approximated below in the median line to its fellow; to these areas the bases of

the labellae are applied. Above and resting between these areas is the concave tip of the labium (seen as a concave band of chitin in section), on which the stylets rest: these three parts enclose a roughly triangular area covered by a delicate membrane, thrown into folds, and extending above along the under surface of the tip of the labium, fusing with its sides and tip; on either side being in connection with the bases of the labellae and with the joint. This membrane bears a few very fine hairs, and it probably allows of considerable play when the labellae are separated; with them it touches the skin when the mosquito sucks blood, being then stretched to some extent.

The labellae are conical and roughly crescent-shaped in section; their apices form the extreme tip of the proboscis. They present two surfaces, an outer convex, an inner concave; and two borders an upper and a lower, the former being in the same line as the edges of the upper surface of the labium.

In some species of *Culex* and in *Anopheles maculipennis* they consist of two parts, a distal, and a basal upon which the distal half is jointed to allow of some outward movement; the joint being represented by a narrow white line beginning near the apex on the outer surface at its upper border and curving sharply downwards and outwards to the lower border; about the centre of this line is a sharp upward bend. This peculiar division of the labella is absent in *Anopheles costalis*. SMITH¹ who points out the homology of the so-called labium of the *Diptera* with the galea of other insects, states with regard to its tip in five species of *Culex* he examined 'no two agreed in structure.' We have found this to be the case also in a few species of mosquitoes we have examined, especially with regard to the structure of the joint at the base of the labella.

The outer surfaces of the labellae are covered with fine hairs and here and there coarser ones. The inner concave surfaces are marked by longitudinally ridges and folds. There are no 'pseudo-tracheae.' In transverse section (plate XV, fig. 1), three regions are distinguished; an upper somewhat flattened, the chitin of which is very thin and thrown into numerous small folds from which arise a felt work of exceedingly fine long hairs, crossing one another in all directions; a lower area of fairly thick chitin limited by the rounded inferior border of the labella: from it arises long thick bristle-like hairs projecting downwards in between the tips of the labellae. These two areas, well marked near the tips of the labellae, gradually fade away towards their bases. Between them, the central region is deeply hollowed out and ridged and folded, its chitin is much thicker and free from hairs. A little below its centre, running longitudinally down this surface is a stout ridge of chitin which can be traced in a cleared specimen of the labium, mounted whole, to the base of the labella (plate XIX, fig. 3): here it makes an outward curve to about the centre of the

1. Smith, *Trans. American Entomol. Soc.*, vol. xvii, 1890, p. 330.

base, and turning sharply back, turns upwards for a short distance to terminate in a rounded knob, which articulates with the chitinous surface described at the distal end of the labium. To the outer bend of this rod the tendon of the muscle of the labella is attached. When these muscles contract, the labellae are drawn apart and rotated in such a way that their inner surfaces look downwards: it is probable that only the anterior distal portion of their inner surfaces is applied to the skin.

The internal structure of the labium. In a section of the proboscis about its middle (plate XV, fig. 1) it is seen that the chitinous exoskeleton of the labium is lined with a delicate spongy tissue containing very large rosette-shaped cells—a continuation of a similar tissue lining the cranium. Beneath the chitinous envelope, here and there, is a row of low cubical epithelial cells (hypodermis). Situated about the centre of the section are the two labial tracheae, one on each side, each surrounded by a delicate cellular sheath; with each runs a comparatively large nerve-trunk—the nerves to the proboscis. The tracheae are the terminal branches of the large tracheae to the head; they join the nerves to the proboscis immediately after their origin from the suboesophageal ganglion: on their way down the labium they give off small lateral branches and becoming smaller, eventually break up into innumerable fine branches about the lower third of the labium to supply the labellae. The nerves, two in number, are the main anterior branches of the suboesophageal ganglion. Running on either side of the common salivary duct they enter the labium beneath the salivary receptacle on the under and outer side of the tracheae, being closely applied to them: after a straight course they split up in the labellae into many fine fibres which are distributed over their inner surfaces.

Internal structure of the labellae. Applied to the outer wall and bulging into the interior of each labella, almost completely filling it is a mass of deeply staining tissue which, with a high power, is seen to be composed of numerous cells very similar in shape and size to the nerve cells of the supra- and infra-oesophageal ganglia of the head. Over the surface of this densely cellular mass, the nerve to the proboscis ends by splitting up into fine filaments (plate XIX, fig. 2). The close relation of the nerve to the proboscis to this structure, points to its being ganglionic in nature, probably supplying the numerous sensory hairs on the inner surface of the labellae with nerve fibres. These ganglia are well supplied with very fine tracheae, the terminal branches of the tracheae to the proboscis.

Muscles of the labium are of two sets:—

Those attached to the base of the labium.

Those arising within the labium.

The latter—the muscles of the labellae—are two long slender paired muscles, each arising by numerous separate bundles of fibres from the dorsal and ventral surfaces of the lateral chitinous ridges of the labium; they are directed very

obliquely towards the tip of the proboscis. These bellies of muscle end in minute tendons which join a very long common tendon, running parallel to the chitinous ridges and extending the whole length of origin of the muscle. These long tendons do not quite reach the mid-line of the labium (plate XIX, fig. 2, *l.m'*); becoming somewhat thicker, they are eventually inserted into the bases of the labellae, chiefly at the chitinous angle mentioned above. These muscles do not appear to take origin from the basal third of the chitinous ridges of the labium. DIMMOCK¹ describes in *Culex* two muscles in relation to each labella, a flexor and an extensor, the flexor being to the inner, the extensor to the other side of the cavities of each lobe, and having origin within the head.

Muscles attached to the base of the labium. One pair of muscles is attached directly to the base of the labium. These are a pair of spindle-shaped muscles, each of which arises from the under surface of the basal chitinous support of the maxilla and is inserted into a ridge of chitin projecting from the groove which separates the labium from the under surface of the head (plate XIX, fig. 1, and XVII, fig. 1, *l.m'*). DIMMOCK describes these muscles in *Culex* as extending along the labium.

The clypeus, or epistom, is the anterior projecting hood-shaped portion of the face from which the proboscis is suspended. It is limited above from the rest of the head by a deep groove; behind and to the right and left of this groove arise the antennae which are to some extent supported by the upper surface, this being slightly hollowed out for the reception of their basal joints; at the sides and posterior are the genae or cheeks, separated from the clypeus by grooves. In transverse section the clypeus appears as a blunt, wedge-shaped piece, the thinner end of which is formed by the upper wall of the pharynx (plate XVI, fig. 2), surrounded below and its sides by a U-shaped area (plate XVII), which for the most part eventually breaks up into the parts forming the proboscis.

From the anterior wall, and from that part of the under surface of the clypeus which forms the roof to the labrum at its origin, project two plates of chitin (endosternites) for some little distance (plate XVIII, fig. 1, *f*): these are approximated below and have an upper and a posterior free edge and two surfaces, inner and outer; the upper posterior angle is lengthened into a blunt process (plate XVI, fig. 2, *f*). These plates are homologous to the fulcrum of other Diptera—for example, *Musca*, *Eristalis*—which have a proboscis capable of extension and retraction. The fulcrum of such Diptera is greatly developed, and moves around an axis at the anterior angle of the head, and encloses the pharyngeal muscles. In the Culicidae the proboscis is fixed in a more or less permanently extended position, and the fulcrum is ill-developed and firmly attached to the anterior wall of the head.

The inner walls of the clypeus afford attachment to three sets of muscles:—

1. Muscles in connection with the labrum.
2. Muscles to the base of the epipharynx.
3. Muscles in connection with the pharynx.

1. Dimmock, *The Anatomy of the Mouth parts, etc., of some Diptera*. Boston, 1881. P. 18.

The muscle attached to the labrum on each side consists of two bundles of fibres lying side by side, having an extensive origin from almost the whole of the upper median surface of the clypeus from before backwards. Their fibres are directed backwards and collect together in a fan-like manner, to be inserted into the projecting chitinous base of the labrum (plate XVIII, fig. 1 and XVII, fig. 1 *lbr.m.*).

The muscles in connection with the base of the epipharynx are two lateral groups arising from the lateral outer wall and free edges of the fulcrum; a few fibres probably arising from the adjacent inner wall of the clypeus. The fibres project vertically downwards, and are inserted into the horizontal plate of chitin on either side of the epipharynx (plate XVI, fig. 2 *e.m.*)

The third set of muscles arise from the upper inner surface of the clypeus on each side of the labral muscle mass; the fibres run backwards and downwards to be inserted into the upper chitinous plate of the ascending portion of the pharynx—each muscle being divided into a central and two lateral portions, inserted into the central membranous and anterior and posterior chitinous portions of the wall respectively (plate XVIII, fig. 1, and XVII, fig. 1 *p.m.*). The remainder of the clypeus is occupied by tracheae and nerves for supply of the above muscles, and it is lined by loose spongy fatty connective tissue.

The pharynx is that part of the alimentary tract, lined with chitin, which extends from the base of the proboscis to the commencement of the oesophagus at the junction of the head and neck. It consists of two portions, a short anterior ascending and a longer horizontal portion, the latter passing through the ganglionic ring formed by the supra- and infra-oesophageal ganglia and their commissures. Here it forms a large chamber—the pumping organ. DIMMOCK¹ describes this part of the pharynx as the oesophagus. The first part of the pharynx is narrow and is a tubular continuation of the epipharynx above and the hypopharynx below; it passes upwards and backwards, ending opposite the furrow separating the clypeus from the head. Here the pharynx suddenly turns backwards and is continued on as the second part of the pharynx. The first part of the pharynx consists of two plates of chitin, an upper and a lower; the former limits the clypeus internally; it is not completely chitinous, in fact only its anterior and posterior portions are chitinised and thin off towards the centre of the plate which consists of a membrane covered with flattened epithelial cells (plate XVIII, fig. 1, and XVII); to this membrane are attached the oblique central fibres of the pharyngeal muscle. On the pharyngeal surface of the anterior chitinous portion of this upper wall of the pharynx are a few low conical papillae (taste papillae) (plate XVIII, fig. 1). The posterior upper edge of this wall is curved slightly outwards upon itself and is attached to the upper wall of the second part of the pharynx by a folded band of chitin.

The ventral wall of this part of the pharynx is a stout plate of chitin, anteriorly continuous with the hypopharynx, posteriorly with the ventral wall of the second

1. Dimmock, *The Anatomy of the Mouth parts, etc.* Boston, 1881. P. 13.

portion. Anteriorly and laterally it is curved upwards, and unites with the sides of the clypeus (plate XVII, fig. 1). From its under surface near its posterior edge it gives off on each side a hook-like ridge of chitin (plate XVIII, fig. 1, α) from which the muscles of the salivary receptacle have origin. DIMMOCK describing the pharynx states 'the channel for the passage of food turns upwards and then backwards again, passing in its course a place where its wall approximate dorsally and ventrally; this narrowing of the walls is probably a valve to prevent the return of fluids to the mouth during the pumping process.'

In *Anopheles costalis*, situated in this position and attached to the upper surface of the slightly horizontally bent posterior end of the ventral chitinous plate, is a peculiar ridge of chitinous stout hair-like processes, which curve forwards so that their tips lie in the angle between the upper surface of the first and second parts of the pharynx. The hairs are of two kinds, an anterior large set—probably a single row—and a posterior, small, fine set situated in a clump immediately behind the former. The larger hairs consist of a short stout shaft firmly embedded in the chitinous pharyngeal wall; this shaft supports a cup with a free rim curved outwards; within the cup lies the oval-shaped bulbous extremity of the base of the hair; this bulbous extremity contains a single large cell. The remaining free portion of the hair curves forwards and tapers to a fine point, and appears to have a central shaft enclosed within a chitinous cuticle from which barb-like processes project. The hairs of the posterior set are much finer and shorter, and are more numerous; they appear to be simple in character. In transverse section (plate XVIII, fig. 2) this structure presents to some extent the appearance of 'rods and cones.' The suboesophageal ganglion lies in close proximity to this structure, but no nerve fibres have been traced to communicate with these specialised hairs, although such probably exist. That in the first place these hairs act in conjunction with the general conformity of this part of the pharynx as a valve to prevent the regurgitation of blood back into the mouth during the action of the pumping organ seems to admit of no doubt; on the other hand such specialisation in structure would lead one to suppose that they possess also a sensory function.

The mechanism of the proboscis. The mosquito, when alighting on the surface of the skin for the purpose of sucking blood, immediately raises the palpi almost at right angles to the proboscis. After probing about with the labellae for a suitable spot to pierce the skin, it plants them firmly on the surface, the proboscis being directed a little forwards. A moment later the labium is seen to bend backwards near its junction with the head, the stylets, remaining straight, becoming thus uncovered. The bending of the labium becomes more marked as the stylets sink into the skin, the angle of the bend travelling towards the middle of the length of the proboscis, so that when the stylets have entered the skin to nearly their full extent, the labium is bent double beneath the head of the insect. REAUMUR was the first, probably,

to describe and figure the manner in which the labium was disposed of during the puncture of the skin. The stylets probably enter the skin as one piece, being guided by the tip of the labium and supported on each side by the basal portions of the labellae. The piercing of the skin is brought about by muscular force directed from the body of the insect, the muscles attached to the bases of the stylets serving to keep them rigid. The withdrawal of the stylets is accomplished by the powerful retractor muscles attached to the chitinous prolongations of the maxillae, and the muscles described in connection with the bases of the other mouth parts. During the process of extraction, while the stylets are slowly sinking into the groove on the upper surface of the straightening labium, the insect keeps the labellae pressed firmly upon the skin. After they have emerged, the labellae spring together over their tips.

By a careful study of the minute anatomy of the proboscis, as detailed above, it is not difficult to suggest a method by which the mature larvae of *F. nocturna* may escape from the proboscis. As above mentioned the dimensions of this larva are 1.006 mm. long and 0.025 mm. broad. It is therefore evident, taking into consideration the dimensions of the several parts of the proboscis, that the most likely method of gaining access to the proboscis from the head is by entering the body of the labium, the structure and disposition of which would easily admit of this. It has been suggested that the larvae lie among the stylets—in which case it will be seen from the study of the attachments of these appendages that the larva would in its course, necessarily have to pierce a stout layer of chitin, a procedure exceedingly improbable. But the evidence that the larvae do reach the labium is now conclusive. Low¹ in sections of the proboscis found them there; and although he describes them as 'making an independent passage through the base of the labium and pushing forward along the proboscis between the labium and the hypopharynx amongst the stylets, where they are found stretched along the length of the proboscis head foremost,' the illustrations of his sections of the proboscis shew the worm in the body of the labium, and he cannot have been intimately acquainted with the minute and most delicate anatomy of these parts. These illustrations certainly do not shew the worm 'amongst the stylets,' but in the tissue of the labium.

GRASSI and NOE² often found *F. immitis* in the labia of mosquitoes (*Anopheles claviger*) which had fed on the blood of an infected dog; and we ourselves, once in a dead mosquito, and again in a living insect, found the larvae alongside the tracheae of the labium.

The question then arises as to how the larva leave the body of the labium and reach man, since it must be presumed that their presence in such an organ as the proboscis indicates that they subsequently leave that organ during or about the time of puncture. Judging from the condition of the larva at this stage, which

1. Low, *British Medical Journal*, 1900. Vol. II, June 16.
2. Grassi and Noe, *British Medical Journal*, 1900. Vol. II, p. 1306.

shows a complete alimentary canal and reproductive apparatus (although immature) similar in site and arrangement to those of the adult worm as found in man, it seems certain the next stage in the life history is carried out in the definitive host—man. It has been suggested, that as mosquitoes can be sometimes observed feeding on such as bananas, that the filariae are capable of exercising a selective instinct for their escape at the time of puncture: and it has been further suggested that possibly the filariae may escape into banana and other food stuffs, and either undergo a further period of their life history in the external world, or without further change be introduced into the alimentary tract of man. All these suggestions appear to us exceedingly improbable. We have previously shewn,¹ and there is a considerable amount of other evidence to support the facts, that a fertilized female mosquito of the blood-sucking species of West Africa requires blood regularly for the maturation of her ova, and that she will have blood and nothing else: and since those species capable of carrying human filaria frequent the neighbourhood of human habitations, they will for the whole period of their existence feed on blood, and generally on human blood—so that the possibilities of the escape of the filariae into banana and other substances are extremely vague, and further, it becomes quite unnecessary to suppose the possession by the larvae of any selective instinct. The occurrence of the larvae in such a position leads one to presume that they leave it before, during or after the act of suction of the blood; and GRASSI and NOE² claim to have infected a dog by the bites of *Anopheles* infected with *F. immitis*, although, since a single broken immature worm only was discovered, *post-mortem*, some sixteen days after the mosquitoes had been allowed to bite them, this experiment urgently requires confirmation. These investigators, however, assert that in specimens of the numbers of mosquitoes which were allowed to bite the dog, before the experiment, larvae were found in their labia, while after the experiment, many labia were dissected and found empty.

GRASSI and NOE in their article go on further and describe how the larvae leave the labium. After drawing attention to the bending of the labium, as the stylets gradually penetrate the skin, so that the angle formed advances from near the base to the middle of the labium until the labium appears almost completely doubled, they 'add the two halves of the olive and the little tongue resting against the skin of the animal, which is punctured, embrace the six pieces penetrating the skin. It is certainly through the bending of the labium, stuffed with filariae, that is brought about the rupture of the integuments of the labium along the dorsal groove, and through the rupture thus produced come out the filariae to penetrate the body of their definitive host. It is difficult, as everyone will understand, to enter into further particulars. In some cases we believe that we positively found the rupture in the middle of the length of the labium in correspondence with the loop. It seems to

1. *Report of Malaria Expedition to Nigeria*, 1901. Part I, chap. iv.

2. Grassi and Noe, *British Medical Journal*, 1900. Vol. ii, p. 1306.

us also that the two halves of the olive and the little tongue being in the above-mentioned position have an importance in directing the movements of the filariae towards the wound made by the stylets. Perhaps the gases emitted in the first moment of the bite help the entry of the filariae into the body of the definitive host.'

One cannot read this paragraph without being struck with the remarkable ingenuity displayed in its account of how the filariae leave the proboscis of the mosquito. But a very careful and exhaustive study of the structure and relations of the parts forming the proboscis has convinced us of the utmost difficulty the most inquiring of observers would experience in deciding the occurrence of any such slit in the upper surface of the labium, as the authors believe they have seen. Furthermore, the upper surface of the labium is composed of chitin almost as thick as that on the outer surface (plate XV, fig. 3). Moreover, from the illustration of the longitudinal section of the proboscis accompanying Low's article, it appears to us that the head of the filaria in the labium is considerably beyond the middle of the labium, in fact appears to reach the distal end of the labium proper—as MANSON¹ says, 'to the tip of the proboscis.' Such a position, if the filariae escape in the manner GRASSI and NOE imply, would necessitate their exit, middle part first, at the bottom of the very acute angle formed by the two almost completely folded parts of the labium. The difficulties involved in such a method of exit appear to us insurmountable.

Referring again to the structure of the extreme tip of the labium (page 80), we have stated that at about 0.16 mm. from the tip of the proboscis the labium proper appears to end bluntly but its upper surface is found to continue on, gradually tapering to a blunt point covered with fine hairs. And again (page 81), above and resting between these areas (lateral areas on the end of the labium proper) is the concave tip of the labium (seen as a concave band of chitin in section) on which the stylets rest; these three parts (the two areas and the concave chitinous band) enclose a roughly triangular area covered by a delicate membrane thrown into folds; above it extends along the under surface of the tip of the labium, and on each side is in connection with the bases of the labellae.

When the tip of the proboscis is applied to the surface of the skin, it has been seen that the two labellae swing apart and are rotated so that their inner surfaces are in contact with the skin, and that the piercing stylets are directed in their course by the concave upper surface of the extreme end of the labium. By the swinging of the labellae the delicate folded membrane is somewhat stretched and is close to the surface of the skin. This membrane is exceedingly delicate so that in transverse section even with the high powers of the microscope ($\frac{1}{12}$ O.E.) the sections of its folded edges are represented by thin fine lines. It will thus be seen that this is the most delicate part of the labium; and as both Low indicates in his illustration and

1. Manson, *Tropical Diseases*, London, 1901. P. 496.

MANSON¹ asserts to have often observed, the head of the mature larva (in fact there appear to be more often a pair) is in the immediate neighbourhood of this spot; it is extremely probable that the larvae escape by the rupture of this thin membrane, which is probably already stretched by their presence, when the labellae swing out, and stretch the membrane still more. The escape of the larvae in this way may possibly be aided as the bend of the labium travels from the base towards the middle of that organ.

The relation between *F. nocturna* and *F. diurna*

The many points of resemblance between the embryos of these two worms suggest the question of their identity, and in favour of the view of their identity many facts can be brought forward. In consequence of the importance of the subject, and the many points of interest involved therein, we propose to treat of the arguments for and against in some detail; and to arrange them under some chief headings.

Geographical distribution. As has been already pointed out, the distribution of elephantiasis (caused by the presence of the adult form of *F. nocturna* in the lymphatic vessels and other sites) is extremely wide; but limiting ourselves to the distribution of *F. nocturna*, as determined by the presence of embryos in the blood, it corresponds in certain regions with that of *F. diurna*—the two occurring side by side throughout large tracts of country. On the other hand, however, there appear to be many lands where *F. nocturna* alone is found; but as far as is at present known, in no district has it been shewn that *F. diurna* prevails alone. Reference must again be made in this connection with the conditions occurring in some of the islands of the Pacific, already mentioned, where elephantiasis is very prevalent, and an embryo occurs in the blood of many natives, which resembles very closely *F. nocturna*, yet shews none of its characteristic periodicity.

The microscopical appearances of the embryos. It has already been stated that in West Africa we were unable to distinguish the embryos in the blood of natives infected with *F. nocturna* and *F. diurna* respectively, by any means whatever. They appeared identical in their appearance, characters, measurements and movements in fresh preparations and correspond in length, breadth, staining reactions, and in the possession of the same number of 'spots,' situated at similar points along the length of the worm and of the same shape and size. The sheath, a common feature of each, appeared identical. Moreover, the West African *F. nocturna* resembles very closely that of China and India as described by MANSON.

The numbers in peripheral blood. Here again there is a close similarity between the two worms. An ordinary case of either infection presents from twenty to sixty embryos in a drop of blood from the finger, at the time when the maximum number is present in peripheral blood—although in each case so many as four to five hundred may be present in exceptional infections.

1. Manson, *Tropical Diseases*. London, 1901. P. 496

Periodicity. It was this phenomenon, and this alone, which led MANSON to regard *F. nocturna* and *F. diurna* as distinct species. And certainly, in the limited condition of the knowledge of the subject, it was a very natural conclusion, one large set of cases which had been examined, shewing a characteristic periodicity with a maximum number of embryos present in peripheral blood at midnight, and a smaller set presenting the reverse conditions, a maximum number at midday. The departure from this interesting regularity to be first noted, was recorded by THORPE in the Tonga Islands where a large percentage of the adults shewed symptoms of elephantiasis, and where an examination of a large number of natives proved the presence of embryos in their peripheral blood both during the day and during the night in approximately equal numbers, and moreover shewed that the embryos were present throughout the whole of the day.

We have already given details of several cases illustrative of the same conditions (table VII), and furthermore we have shewn (tables VIII and X) that cases of filarial infection occur in whom the hour at which the maximum number of embryos is present in peripheral blood is not mid-day and midnight, but may be any other hour—3, 6, or 9 a.m. or p.m. And besides we have shewn that 'pure' cases of *F. diurna* and *F. nocturna* are considerably less frequent in West Africa than these irregular cases.

The definitive hosts. THORPE, probably bearing in his mind the classical experiment of MACKENZIE, and the repetition of that experiment in another case by MANSON, by which it was proved that by a change in the habits of a case of *F. nocturna*, the periodicity of the embryos could be completely inverted, becoming thus similar to that of *F. diurna*, explained the peculiar phenomenon of the occurrence of the embryos in the blood of the natives of the Friendly Islands by the habits of the natives, which he thus describes from MARINER's classical account of the Tonga Islands :

'The natives employ themselves in conversation not only at any time during the day but also at night. If one wakens, and is not disposed to sleep again, he wakens his neighbour to have some talk. By and by, perhaps they are all aroused, and join in the conversation. It sometimes happens that the chief has ordered his cooks in the evening to bake a pig or some fish and bring it hot in the middle of the night with some yams. In this case the torches are lighted, and they all get up to eat their share, after which they retire to their mats ; the torches are put out, some go to sleep, and others talk perhaps till daylight.'

Similar habits are in practice among the natives of the whole of West Africa, but to a larger extent and on a larger scale. We were often told by natives from different parts of the Coast that it is common practice in the respective countries to which they belong, to sing and dance the whole night through, especially on moonlight nights. In fact we have ourselves heard the midnight orgies in the native

towns which we visited, and especially of the Kroo boy gangs in the towns of Southern Nigeria. Moreover, we often observed, especially in those towns where civilisation was very backward, the natives asleep during the middle hot part of the day; indeed, the Kroo boy in English Government employ steals a mid-day nap whenever he can. These habits have been practised, no doubt, for generations, and probably were prevalent to a much greater extent for years before the influence of Europeans was felt. Such conditions would, in a great measure, account for the variety in the cases of filarial infection we met with in West Africa, and which THORPE observed in the Friendly Islands, and point strongly to the identity of the two embryos, or rather to the phenomenon of the accommodation of the one or the other or of an original embryo perhaps exhibiting no periodicity whatever, to the varying habits of the natives who formed their habitat.

The intermediary host. *F. nocturna* has been successfully cultivated in several species of mosquitoes of both genera. In West Africa, after several attempts, we were able to cultivate this embryo in *Anopheles costalis*; but all our efforts to cultivate *F. diurna* failed. But this is not remarkable, for, if *F. diurna* had been evolved in consequence of the habits of the natives, it is not unnatural to expect that its intermediary host is an insect, probably a mosquito, not essentially nocturnal in its habits such as *A. costalis*, but one whose habits are diurnal.

Analogy with avian filariasis. In the chapter on Avian filariasis we describe eleven new species of filariae, each having a different embryo; in fact, we were soon able after a little practice to decide the species of the worm even by a study of the stained specimen of the embryo. Each species then possesses distinct adults, which give rise to a characteristic embryo. This would suggest a similar condition among human filariae, and thus that *F. diurna* and *F. nocturna*, being indistinguishable in fresh and stained specimens, have a common adult form.

The adult form. The adult of *F. nocturna* is well known—*F. bancrofti*. The adult of *F. diurna* has not yet been described, unless *F. loa* be that form. Now, the distribution of *F. loa* is, as far as we can ascertain, limited to the West Coast of Africa, and MANSON makes the same statement. It has not been met with in any other part of the world,* and the occurrence of a worm of the length of *F. loa* occurring under the conjunctiva of the eye, cannot possibly have been overlooked anywhere.

F. diurna, as far as we at present know, is also apparently limited to the West Coast of Africa, and has been found in some cases of natives in which *F. loa* has been removed from the eye—although this is not remarkable as anything more than an ordinary coincidence, considering the prevalence of *F. diurna* cases on the Coast. Moreover cases of *F. loa* have occurred in which no embryos could be demonstrated in the blood.

* Stossich states that it occurs in the Antilles and Guiana, but Manson says, in his latest edition of *Tropical Diseases*, 1900, 'it is peculiar to the West Coast of Africa.'

The conditions in the Friendly Islands, previously often referred to, may perhaps be quoted as an exception to the statement above—that *F. diurna* is limited in its distribution to West Africa—since the embryos cannot be regarded as nocturnal. Probably this condition will be found to be much more extensively distributed. On the other hand we have described the embryos of *F. loa* as very similar to those of *F. nocturna*: but on closer study some points of difference may be noted in the disposition and number of the spots. Such a close resemblance indicates either that they are identical with *F. diurna* and that, therefore, *F. loa* is the parent form of *F. diurna*, or that, being very much alike in all other respects except in the matter of the spots as just mentioned, they are intended for a more or less similar life history in their intermediary hosts.

To sum up, although the weight of evidence is on the side of the identity of *F. nocturna* and *F. diurna*, there are many points which remain to be cleared up before the question can be settled. The *F. loa* has introduced a serious difficulty into the subject, and it appears to us that a solution of the mystery can only be obtained when the embryos in a pure case of *F. diurna* have been successfully and completely cultivated in their intermediary host—which is still to be discovered—to the final larval stage, and perhaps it may become necessary to perform experiments of infection of man by the use of infected intermediary hosts before a complete solution is procured.

APPENDIX

NOTES ON A COLLECTION OF MOSQUITOES FROM WEST AFRICA, AND DESCRIPTIONS OF NEW SPECIES

BY F. V. THEOBALD, M.A., F.E.S., ETC.

The collection of mosquitoes brought back by the members of the expedition from the West Coast of Africa contained twenty-six distinct species. Of these only five had been previously described. Thirteen of the new species are described in my Monograph of the *Culicidae* shortly to be published by the Trustees of the British Museum, and the remaining new ones here.

The collection includes the genera *Anopheles* Meigen (two species), *Mucidus* Theobald (one species), *Eretmapodites* Theobald (one species), *Stegomyia* Theobald (five species), *Culex* Linnaeus (nine species), *Panoplitus* Theobald (one species), *Taeniorhynchus* Arribalzaga (modified) (two species), *Aedes* Meigen (one species), *Uranotaenia* Arribalzaga (three species).

The collection contained over two hundred and fifty specimens, including two midges (*Chironomidae*). Some of the types have been given me by the collectors for the British Museum.

GENUS *Anopheles*. MEIGEN (1818)

(*Syst. Besch. Eur. Zweifl. Ins.* p. 1-13, 1818)

I. *Anopheles costalis*. LOEW

(*Berlin Ento. Zeitschr.* p. 55, 1866)

A number of this species taken at Bonny, Duke Town, Bugama, Bakana, Akwete Prison, s.s. Sobo (off Bakana), Lokoja, and at Old Calabar. They show considerable variation both in colour and size, but the costal markings and the spots on the femora remain distinct in all the specimens. Those from Old Calabar are considerably paler and somewhat smaller than those from Bonny. The specimens also show considerable variation in leg banding, it being almost absent in some, very distinct in others.

They were captured during the following months—April, in Duke Town; May and June, at Bonny; August, at Akwete; September, at Lokoja; in June, off Bakana; and June at Opobo.

II. *Anopheles barbirostris*. VAN DER WULP var. *Africanus*

(*Leyden Museum Notes*, VI, p. 48)

Three dark ♀ *Anopheles* taken at Old Calabar in April are undoubtedly this species. They resemble in all structural respects the Asiatic form. The only difference to be noticed is that some pale scales are scattered over the wings, and there are no traces of leg banding. There is nothing upon which a new species could be founded, but they are certainly a local variety, and they look longer-legged than the Malay and Indian specimens I have seen.

The examination of the ♂ unguis *might* prove it to be quite distinct. I propose to call it variety *Africanus*; the variety based solely on the mottled wing scales.

GENUS *Mucidus*. THEOBALD (1901)

(Mono. Culicidae, Vol. I)

A single species of this genus occurs in the collection, represented by five specimens.

The characters of the genus *Mucidus* are as follows :—head clothed with narrow curved, forked upright, and *long twisted scales*. Thorax with narrow curved scales and long twisted ones, which are apically expanded. Abdomen densely scaled, the scales giving it a ragged appearance, Legs banded, densely scaled with projecting scales; fore and mid unguis of the ♂ unequal, the larger with two, the smaller with one tooth; hind unguis equal, small, toothed; in the ♀ all the unguis are equal, very thick, uniserrated.

Wings covered with broad pyriform scales, many parti-coloured. Antennae 14-jointed in ♀. Palpi of ♀ half as long as the proboscis; of the ♂ 6-jointed, a little longer than the proboscis. The venation of the wing is much as in *Culex*, but the posterior cross-vein is nearer the apex of the wing than the mid cross-vein. The insects have a mouldy appearance, due to the long twisted scales. The genus occurs in Australia, East Indies, Malay Peninsular, and the West Coast of Africa. They are often vicious biters.

Mucidus africanus. THEOBALD

(Mono. Culicidae, Vol. I)

Five specimens of this distinct species were taken at Asaba in August, and can at once be recognized from other West African mosquitoes by the densely scaled legs and ragged appearance of the body.

GENUS *Eretmapodites*. THEOBALD (1901)

(Mono. Culicidae, Vol. I)

Two species of this genus only occur. The genus *Eretmapodites* is founded on a West African form in which the head is clothed with flat and upright-forked scales, there being no curved scales as seen in *Culex*. The palpi of the ♀ are 4-jointed; in the ♂ 5-jointed, long and thin, pointed, and with no hair tufts; the mesothorax clothed with narrow, curved, hair-like scales, and the scutellum with flat scales on the mid lobe. *The last two tarsi* in the ♂ are densely scaled, forming a distinct tuft (Fig. 1, Pl. I) in one species. The fore unguis of the ♂ are unequal, the larger simple, the smaller uniserrated; the larger one stout, the smaller thin; mid unguis unequal and simple. Venation much as in *Culex*.

IV. *Eretmapodites quinquevittata*. THEOBALD

(Mono. Culicidae, Vol. I)

A single female of this species from Duke Town, Old Calabar, was taken in May. It is rather damaged and presents no peculiarities. It also occurs at Sierra Leone. The species can easily be told by the ferruginous thorax, with dark longitudinal lines, the abdomen almost black with silvery, oblique, lateral, shining spots and the densely scaled two hind apical tarsi of the ♂ (when fresh). The other species of the genus *E. Austenii mihi* has the tarsal paddle absent.

GENUS *Stegomyia*. THEOBALD (1901)

(Mono. Culicidae, Vol. I)

Differs from *Culex*, in that the head and scutellum are both covered entirely with flat scales, that former having a few upright-forked ones as well. Palpi short in the ♀; long in the ♂, apparently five-jointed in the latter, and generally nude. Abdomen banded or plain, but with lateral spots. Fork-cells of the wing rather small. Eggs usually laid singly, not in rafts.

V. *Stegomyia fasciatus*. FABRICIUS (1805)*S. taeniatus*. WIEDEMANN (1828), ETC., ETC.

(Syst. Autl. 36-13)

This common mosquito, which occurs between latitude 30° N. and 30° S., is evidently abundant in West Africa, specimens in this collection coming from Old Calabar and Bonny. They were captured chiefly in April, May, and July. The majority are rather small specimens, and some of them show the abdominal banding involving both sides of the segments. This species occurs right into Central Africa, and is, perhaps, the commonest tropical and sub-tropical mosquito, biting during the day as well as at night.

It can easily be told by the thoracic ornamentation; the insect is very dark-brown to black, the bases of the abdominal segments with creamy-white bands and white lateral spots, the legs basally white banded, and the thorax with tawny to brown tomentum (scales), with a silver curved line on each side, two narrow parallel ochraceous or yellow lines in the middle and some silvery-white scales on the scutellum, forming a line of three spots. The majority of the specimens in this collection show a pure white line of scales on each side of the space in front of the scutellum, which I have not noticed so plainly before.

VI. *Stegomyia africanus*. THEOBALD

(Mono. Culicidae, Vol. I)

Two ♀'s; one from Duke Town, one from Bonny; taken in April and May.

It is very like *S. fasciatus* Fab., but has two lateral oblique silvery side bars to the mesothorax, no central ornamentation, except a silvery spot in front, and the second tarsal joint of the hind legs is nearly all white. Abdomen generally quite devoid of banding, but one specimen shows faint traces of basal fascia. Giles' *S. gubernatoris*, from India, is very similar but quite distinct.

This mosquito occurs in Central Africa as well as on the West Coast.

VII. *Stegomyia irritans*. Nov. sp.

(Fig. 2, Pl. I)

Head black and grey, the black forming a triangular patch on each side. Thorax chestnut-brown, with deep-brown, and bright scanty golden scales. Abdomen dark-brown, with narrow, basal, white bands. Legs dark-brown, unbanded.

♀. Head covered with flat, creamy, grey and black scales, the black ones forming a more or less triangular patch on each side and a small area in the middle, a few scattered, black, upright-fork scales over the occiput, around the eyes a narrow line of curved, golden scales; clypeus

black, apparently nude ; palpi testaceous, with dark scales ; antennae dark-brown, with narrow, pale bands, basal joint half testaceous, the inner half darker, base of the second joint testaceous, basal joint with a few small scales on the inner side, and minute curved hairs ; proboscis deep brown ; eyes black and golden.

Thorax deep chestnut-brown, with narrow, curved, deep-brown scales and ornamentation of similar bright golden ones, the latter most prevalent over and in front of the roots of the wings. Scutellum brownish, with flat, black scales on the middle lobe ; narrower, rather curved, creamy ones on the lateral lobes, and with deep-brown border-bristles ; metanotum brown ; pleurae brown, with large patches of creamy scales.

Abdomen deep blackish-brown with narrow, white, basal bands, first abdominal segment rather ochraceous, covered with dusky-black scales and pale-brown hairs ; posterior border-bristles chestnut brown, alternately long and short ; venter mostly creamy white with narrow dark apical bands to the segments ; the dorsal white bands form more or less white lateral spots.

Legs dark brown, pale at the base, femora grey ventrally ; femora, tibiae, and metatarsi spiny ; fore and mid unguis equal uniserrated, hind equal and simple.

Wings with the fork-cells rather short ; scales brown ; first submarginal cell very little longer and slightly narrower than the second posterior cell, their bases about level, stem of the former equal to about half the length of the cell, of the latter nearly two-thirds of its length ; posterior cross-vein a little more than its own length distant from the mid cross-vein.

Halteres ochraceous, with pale scales over the knob, and dark ones on one side.

Length.—3 mm.

♂. Antennae black, with dense black plumes ; palpi pale ochraceous, densely covered with black scales, the antepenultimate joint with two narrow pale rings ; apical joint small, a little more than half the length of the penultimate joint, acuminate, penultimate joint wider than the apical, the antepenultimate expanding at the tip, the last two with long, brown hair tufts on one side, especially the penultimate, a few long hairs on the apex of the antepenultimate, and a few long black bristles on the apex of the last two joints ; proboscis deep brown, almost black. Fore and mid unguis unequal, the larger uniserrated ; hind unguis equal, small and simple. Fork-cells of wings small ; the first submarginal cell shorter and considerably narrower than the second posterior, its stem nearly equal to the length of the cell ; stem of the second posterior cell equal to the length of the cell.

Length.—4 mm.

Habitat.—Bonny.

Time of Capture.—May.

Observations.—Described from a series of ♀'s and a single ♂ in the collection of the Expedition. It is a clearly defined species, with banded abdomen and unbanded legs. The deep chestnut-brown thorax and grey and black head and unbanded legs separate it at a glance from all other African *Stegomyias* I have seen, except *S. nigeria*, from which it differs in thoracic ornamentation, the two parallel pale lines on the mesothorax of *S. nigeria* being absent.

VIII. *Stegomyia nigricephala*. Nov. sp.

(Fig. 3, Pl. I)

Head entirely black. Thorax dark-brown, with bronzy-brown scales. Abdomen black, with small, white, basal, lateral spots. Wings with dark-brown scales, and slightly tinged with brown. Legs dark-brown, unbanded.

♀. Head black (Fig. 3b, Pl. I), entirely covered with flat, black scales; clypeus, proboscis, and palpi black; antennae dark-brown; basal joint testaceous on one side, dark on the other; eyes golden.

Thorax black, with rather long, bronzy-brown curved scales, forming a dense matting over the black surface; over the roots of the wings numerous jet-black bristles; scutellum testaceous in the middle at the base, black at the apex, lateral lobes greyish-brown, mid lobe with flat black and grey scales and six (?) black border-bristles; metanotum blackish; pleurae very dark, with three large patches of white scales.

Abdomen (c) testaceous at the base, steely-black apically, covered with black scales, each segment with a small, basal, white, lateral spot; venter black, with basal white bands.

Legs dark blackish-brown, coxae and trochanters pale-brown; fore and mid (d) unguis equal, biserrated, hind equal and simple.

Wings slightly tinged with brown; veins clothed with dark-brown scales; fork-cells small, the first sub-marginal cell a little longer but no narrower than the second posterior cell, its stem equal to about two-thirds of the length of the cell; stem of the second posterior as long as the cell; posterior cross-vein nearly twice its own length distant from the mid cross-vein.

Halteres with deep ochraceous stem and fuscous knob.

Length.—4.8 mm.

Habitat.—Bonny.

Time of Capture.—May.

Observations.—Described from a single ♀. The specimen was taken from a native hut. It can at once be told by the entire covering of black scales on the head and the rather long, curved, bronzy scales on the thorax and the unbanded abdomen.

GENUS *Culex* L. (1735)

(*Syst. Nat.* 1735)

Palpi short in the ♀, long in the ♂, apical joint of latter usually acuminate, but sometimes clavate. Head clothed with narrow curved, upright-forked and broad flat lateral scales; scutellum covered with narrow curved scales; those on the thorax in three forms, narrow curved, narrow hair-like curved, and flat spindle shaped. Wings having the lateral vein scales linear, as a rule, and the first submarginal cell generally longer and narrower than the second posterior cell.

Eggs laid in rafts.

I have still retained several species in this genus which will have to be removed later.

IX. *Culex duttoni*. Nov. sp.

(Fig. 4, Pl. I)

Thorax dark-brown with golden-brown to golden narrow curved scales, with pale scaled areas in front, over the wings, two pale spots and pale scales in the middle of the back of the mesonotum, continuous with those over the wings. Abdomen with basal creamy-white bands. Legs with banding involving both sides of the joints.

♀. Head dark-brown with narrow creamy curved scales around the eyes, on the back of the occiput and in the middle, those between of a more golden-brown hue; the upright fork scales in front (forming a band around the head) bright brown, those behind creamy, at the sides of the head are a few small white flat scales; the fork scales are very numerous, there is also a row of bright-brown bristles projecting forwards over the eyes; clypeus black; palpi black scaled with a few

pure white ones up one side ; proboscis deep blackish-brown, apex testaceous and with a dull testaceous band on the apical half ; antennae deep-brown. Thorax black, covered rather densely with narrow golden-brown curved scales, and pale rather broader creamy ones arranged as follows :—around the front of the mesothorax, forming a narrow line, a more or less distinct spot on each side about the middle of the mesonotum, a long patch just over the roots of the wings, which bend round and pass up again on to the mesonotum, these latter are almost white ; scutellum brown with narrow curved pale-golden scales, eight median golden-brown border-bristles, with some smaller fine pale golden ones over them ; metanotum deep-brown ; pleurae dark-brown with a few small patches of white scales.

Abdomen (*d*) deep-brown with basal dull creamy-white curved bands, and with more or less evident small lateral and basal pure white spots ; border-bristles rather long, lateral ones also long.

Legs with the coxae and trochanters ochraceous ; femora deep-brown, pale, almost white beneath, apex white ; tibiae brown, with slightly paler base and apex, and with pale hairs ; metatarsi with the apex pale banded, fore tarsi with the first and second joints apically and basally pale banded, the third basally banded, the fourth only showing a trace of basal banding. Mid tarsi the same as the fore ; hind tarsi also very similar ; unguis small, equal, and simple ; hind metatarsi longer than the hind tibiae.

Wing with typical brown *Culex* scales ; fork-cells rather long ; first submarginal cell longer and narrower than the second posterior cell, its base nearer the base of the wing, its stem rather less than one-third the length of the cell ; second posterior cell with its branches slightly contracted where they join the wing, its stem rather less than one-half the length of the cell ; posterior cross-vein nearly twice its own length distant from the mid cross-vein. Halteres pale ochraceous.

Length.—4.8 to 5 mm.

♂. Palpi (*c*) dark-brown, with five white broken bands, last two joints with black hairs ; apex of the antepenultimate also slightly hairy, apical joint acuminate ; proboscis deep-brown, with an indistinct pale band ; antennae dark-brown, with deep-brown plumes, faintly banded paler brown ; basal joint deep ferruginous.

Abdomen narrow, the basal creamy-yellow bands prominent. The last segment with creamy-white scales in the middle ; abdomen hairy. Legs banded much as in the ♀, but the last two tarsi seem to be unbanded ; fore unguis unequal and uniserrated ; hind equal, simple and small ; wings with the fork-cells very small, first sub-marginal very little longer, not much narrower than the second posterior, its base nearer the apex of the wing than that of the second posterior cell, its stem slightly longer than the cell, posterior cross-vein about its own length distant from the mid cross-vein.

Length.—5 mm.

Habitat.—Duke Town.

Time of Capture.—April.

Observations.—Described from a series bred from larvae obtained at Canoes Creek, Duke Town. The thoracic ornamentation soon loses its characteristic appearance by denudation, the golden scales only remaining ; the tarsal banding involving both sides of some of the joints and the faintly-banded proboscis should readily separate it from other African species. The banding on the abdomen in the male spreads out laterally in the sixth and seventh segments. The two, sometimes three, white bands on the antepenultimate joint of the male palpus are very characteristic, the most apical band being very wide.

X. *Culex decens*. Nov. sp.

(Fig. 5, Pl. I)

Thorax deep-brown to black with chestnut-brown scales; abdomen almost black with basal uniform white bands on the third to fifth segments, which widen out prominently on the sixth and seventh to form clear lateral spots. Legs dark-brown unbanded.

♀. Head almost black with small narrow curved creamy scales, and numerous dark upright-forked scales, quite black in some lights, the pale scales form a distinct line round the eyes; clypeus dark-brown; palpi deep-black; antennae dark-brown with black verticillate hairs and pale pubescence; proboscis deep bronzy-brown.

Thorax black, deep-brown in some lights, with very narrow curved bright chestnut-brown scales, rather paler in front, two dark median parallel lines show on the denuded surface; bristles deep-brown, especially long and thick over the roots of the wings; scutellum brown with very small narrow curved pale scales, seven bright-brown chaetae to the mid lobe; metanotum brown; pleurae ochraceous and slatey grey, with two patches of white scales, and an elongated patch just over the first two pair of legs.

Abdomen covered with deep blackish-brown scales, the first segment dull ochraceous with two median patches of dull-black scales and long pale hairs, the second to fifth segments with basal white bands, in the fifth the band spreads out a little laterally, on the sixth and seventh the band is rather broken in the middle but much expanded laterally, the eighth segment mainly white; border-bristles longest at the sides.

Legs brown, unbanded, coxae to base of femora pale, venter of femora grey, remainder deep-brown, femora, tibiae and metatarsi, especially of hind legs spiny; unguis small, equal, curved, simple.

Wings with the veins with typical brown *culex* scales; first long vein rather bent about half way along the wing; first submarginal cell longer and just slightly narrower than the second posterior cell, its base nearer the base of the wing, its stem equal to about one-third of its length; stem of the second posterior cell equal to about half the length of the cell; posterior cross-vein nearly twice its own length distant from the mid cross-vein; halteres with ochraceous stem and fuscous knob.

Length 5mm.

♂. Palpi all deep-brown to dull-black, just a trace of a narrow pale band near the base, the apical joint a little longer than the penultimate joint, acuminate, the two last joints with numerous blackish hairs, short and dense on the under surface only, a few also at the apex of the antepenultimate joint, the remainder with short, pale hairs all on the ventral surface, densely scaled below; the palpi are longer than the proboscis by the last joint and the apical third of the penultimate joint; proboscis dark-brown, apex testaceous; antennae grey, with deep-brown bands and brown plume-hairs.

Thorax as in the ♀; abdomen narrow, ornamented as in the ♀. Legs unbanded, traces of a pale knee spot; fore and mid unguis unequal, uniserrated; hind unguis small, equal.

Length.—4.5 mm.

Habitat.—Bonny.

Time of capture.—May.

Observations.—Described from a single ♂ and ♀ in the collection. The abdominal banding of the seventh and eighth segments expanding laterally, serves as a good means of identifying it at a glance.

XI. *Culex maculicrures*. (THEOBALD)

(Mono. Culicidae, Vol. I)

Four specimens (two ♂'s and two ♀'s) of this large brown species, bred from larvae taken at Bonny, and hatched during June. The ♀ measures between six and seven mm.; the thorax is dark-brown, with reddish-brown scales, and shows two prominent pale spots, with a pale line running from each backwards, and sometimes one or two pale and indistinct spots in front. The abdominal segments have narrow, apical, dull-yellow borders. The legs are brown and unbanded, but the femora and tibiae have a row of small yellow spots on one side.

This mosquito has a wide distribution in Africa, and Dr. BANCROFT has recently sent it from Australia (Queensland).

XII. *Culex metallicus*. THEOBALD

(Mono. culicidae. Vol. I)

(Fig. 14, Pl. III)

A number of this very distinct and pretty species, both ♂'s and ♀'s, taken during July, in the Bush opposite St. Stephen's Cathedral, Bonny.

It can at once be told by the thorax being silvery on the front half, brown on the posterior half, and by the more or less brilliant metallic violet abdomen, which is unbanded, as also are the legs, the femora being silvery at the base.

I have not seen this species from any other district in Africa, but I have the remains of a species very similar to it from Siam. It is only provisionally placed in *Culex*.

XIII. *Culex pruina*. Nov sp.

(Fig. 6, Pl. I, and Fig. 7, Pl. II)

Thorax covered with frosty-grey scales, with traces of two parallel darker lines; abdomen with the fifth to eighth segments with basal lateral white spots, almost forming bands, bases of the other segments slightly paler, in the ♂ with more or less distinct banding. Legs brown, unbanded.

♀. Head brown, clothed with hoary, narrow curved scales, and numerous ochraceous upright-forked ones; eyes black; clypeus, palpi, and proboscis deep-brown; antennae brown, basal joint paler.

Thorax shiny black, covered with thin, hair-like, curved hoary scales, and showing traces of two dark parallel bands on the denuded surface; scutellum with narrow curved hoary scales; metanotum testaceous and ochraceous; pleurae dark-brown above, ochraceous below. Abdomen (Fig. 6, Pl. I) dark-brown, almost black; the fifth to eighth segments with basal white lateral patches, which are most pronounced on the sixth, seventh, and eighth segments; the abdomen shows violet reflections; border-bristles pale. Legs brown, unbanded, ventral surface of the femora nearly white; unguis equal and simple.

Wings (Fig. 7a, Pl. II) with pale-brown, typical *Culex* scales; fork-cells rather long and narrow, the first submarginal longer, but no narrower than the second posterior cell, its base nearer the base of the wing than that of the latter; its stem about one-fourth the length of the cell. Stem of the second posterior cell about one-half the length of the cell; supernumerary cross-vein long and sloping, forming a very acute angle with the mid cross-vein; posterior cross-vein longer than the mid, and about one-and-a-half times its own length distant from it. Halteres ochraceous.

Length.—5 to 5.2 mm.

♂. Palpi ochraceous, covered with dark-brown scales, a small pale band near the base, the last two joints with dense black hairs, and also on one side of the apex of the antepenultimate joint; antennae banded, brown and grey, with deep flaxen-brown plumes; proboscis deep brown, apex testaceous. Abdomen narrow, expanding apically, the fourth and fifth segments with basal white bands, the sixth, seventh and eighth with pale bands expanded laterally, the ninth mostly white, moderately hairy. Fore and mid unguis unequal (Fig 7*b*, Pl. II), the larger one uniserrated, the smaller with a tooth near the base, hind equal and simple.

Length.—5 to 5.3 mm.

Time of capture.—August.

Habitat.—West Africa.

Observations.—Described from five specimens in this collection. A very distinct species with hoary scaled thorax, which has a dull golden tinge however in some lights, the banding of the abdomen and the form of the cross-veins are also characteristic.

XIV. *Culex invenustus*. Nov. sp.

(Fig. 8 and 9, Pl. II.)

Thorax dark-brown; abdomen black, unbanded and unspotted. Legs dark-brown, with pale-grey bases, fore and mid femora thick.

♀. Head (Fig. 8*b*, Pl. II) almost black, with narrow ochraceous-grey curved scales, blackish and brown, thin, upright-forked ones, flat white scales at the side, and a narrow white border round the eyes; eyes black; palpi short, dark-brown; proboscis rather short, dark-brown testaceous at the tip; antennae dark-brown, basal joint black, last two joints very hairy; clypeus black; thorax dark steely-black, covered with small, dull bronzy-brown, flat scales, forming a complete layer; when denuded the thorax shows three narrow parallel black lines; scutellum greyish-brown, with narrow curved pale scales and black border-bristles; metanotum dark-brown; pleurae ochraceous brown, slightly darker in front.

Abdomen deep blackish-brown, narrow, unbanded and unspotted; posterior border-bristles dull-brown; venter rather pale.

Legs unbanded, deep-brown, coxae pale, fore and mid femora (Fig 9, Pl. II) swollen, hind femora narrower, pale beneath, tibiae and metatarsi rather bristly; unguis small, much curved, equal and simple. Wings with brown scales of typical *Culex* form; fork-cells moderately long, the first sub-marginal considerably longer, but no narrower than the second posterior cell, its stem about one-fourth the length of the cell, its base nearer the base of the wing than that of the second posterior cell, stem of the latter, half the length of the cell; posterior cross-vein nearly twice its own length distant from the mid cross-vein.

Length.—3.5 mm.

Time of capture.—June.

Habitat.—Degama, West Africa.

Observations.—Described from a single perfect ♀. It can at once be distinguished by the general brown colour, unbanded and unspotted abdomen, and by the swollen fore and mid femora. It comes very near my *Culex longipes* in appearance.

The much swollen femora are probably of generic value, but I have only seen two specimens, both ♀'s, showing this character, and hence place them provisionally in *Culex*. *C. longipes mihi* comes from the Malay Peninsular.

XV. *Culex nebulosus*. Nov. sp.

(Fig. 10, Pl. II)

Head dark-brown with a pale border round the eyes. Thorax brown with tawny-brown scales. Abdomen dark-brown with traces of dull, grey apical lateral spots. Legs unbanded.

♂. Head dark-brown with narrow, curved, dull golden-brown scales, numerous brown, upright-forked ones, and a distinct white border round the eyes, and white scales at the sides; clypeus, proboscis, palpi, antennae brown, basal joint of the latter testaceous at the base; eyes black and golden.

Thorax shiny-black, covered densely with very narrow, curved, tawny-brown scales, and showing two darker parallel lines on the denuded surface, numerous golden-brown and dark-brown bristles over the roots of the wings; scutellum brown with very narrow, almost hair-like, pale scales, seven bristles to the mid lobe; metanotum dark chestnut-brown; pleurae brown and ochraceous with scanty flat white scales.

Abdomen deep-brown, unbanded, with dull violet reflections, indistinct apical, creamy-white lateral spots (Fig. 10c, Pl. II); venter grey and brown.

Legs brown, unbanded; coxae and trochanters ochraceous, the former with dull white scales; femora dull, pale ochraceous beneath.

Wings (Fig. 10a, Pl. II), with brown scales of typical *Culex* form; first submarginal cell considerably longer and a little narrower than the second posterior cell, its stem less than one-third the length of the cell; stem of the second posterior equal to about half the length of the cell; posterior cross-vein considerably longer than the mid cross-vein, about its own length distant from it.

Halteres with slightly fuscous knob and ochraceous stem.

Length.—3 to 3.5 mm.

Time of capture.—April, August, September.

Habitat.—Old Calabar.

Observations.—Described from six specimens. A rather obscure species, with traces more or less distinct of pale, apical, lateral, abdominal spots, and rather marked cephalic ornamentation.

XVI. *Culex fatigans* WIED (1828)

(Ausseurop, Zweifflug Ins. p. 10)

This common mosquito also occurs in West Africa, but is only represented in the collection by a single ♀. It does not seem common, however, in this part of Africa judging from the collections I have received from Bonny and the neighbourhood, but, perhaps, owing to its commonness, it has not been collected. Like *S. fasciatus* Fab. its distribution is very wide, and it is one of the most troublesome species, biting chiefly at night and acting as one of the *Filaria* carrying hosts.

It closely resembles *Culex pipiens* L. of Europe and North America, but it can always be told by the stem of the first submarginal cell being relatively much longer than in *C. pipiens*. The stem in *C. pipiens* is never less than one-fifth the length of the cell, in *C. fatigans*, it is always more, often only one-third the length.

XVII. *Culex rima*. Nov. sp.

(Fig. 11, Pl. II)

Thorax deep-brown. Abdomen deep-brown, with metallic-bronze and violet reflections, white, apical, lateral spots and grey venter. Legs deep-brown, unbanded. Wings with rather broad scales like *C. atratus*, THEO. Ungues small, curved, equal, and simple.

♀. Head dark-brown, with narrow, curved, dull-grey scales and numerous short, upright, black ones; clypeus (*b*) black, with a transverse sulcus; antennae brown, with reddish-brown basal joint; proboscis black, testaceous at the apex; palpi rather thick, black.

Thorax deep-brown, with very minute, narrow curved, dull-brown scales and long black, backwardly-projecting bristles; scutellum deep chestnut-brown in the middle, greyish apically, with narrow dull-brown curved scales and black border-bristles; metanotum deep-brown; pleurae greyish or greyish-brown.

Abdomen bronzy-black, with deep bronzy-green and deep-violet reflections when held in different lights, the four posterior segments with *four distinct, white, apical spots*; posterior border-bristles dull-brown, short; apex pubescent. Legs deep-brown; the coxae very pallid, and also the venter of the femora; the metatarsi and tarsi with somewhat dull, ochraceous reflections ventrally. Ungues small, equal, and simple.

Wings (Fig. 11a, Pl. II) densely scaled towards their apices with rather short, thick, brown scales (*a*¹) (as in *C. atratus* THEO.); fork-cells rather short, first submarginal cell longer and narrower than the second posterior cell, their bases not nearly level, that of the former, nearer the base of the wing; stem of the first submarginal equal to about half the length of the cell; stem of the second posterior as long as the cell; posterior cross-vein slightly curved in the middle, nearly three times its own length distant from the mid cross-vein; fringe brown, very dark at the apex of the wing.

Halteres with ochraceous stem and fuscous knob.

Length.—2·8 mm.

Habitat.—Old Calabar.

Time of capture.—April.

Observations.—Described from three ♀'s. A small species with very distinct abdominal ornamentation. In two specimens the thorax is paler brown. It is closely related to the little black *Culex* I call *Culex atratus*, common in Jamaica. The peculiar wing scales and general facies of these two species will necessitate their removal from *Culex*, but I am waiting for more material as I have only received one damaged ♂ (*C. atratus*) of this group.

XVIII. *Culex invidiosus*. Nov. sp.

(Fig. 12, Pl. II)

Head deep-brown with greyish sheen, seen in some lights; thorax deep chestnut-brown; abdomen blackish-brown, unbanded and unspotted; pleurae paler brown; legs deep-brown, coxae and bases of femora pale.

♀. Head deep-brown, almost black, covered with dull ochraceous grey narrow curved scales over the occiput, black upright-forked ones, and small flat dull white lateral ones, a narrow, rather indistinct grey border round the eyes; clypeus deep chestnut-brown; proboscis deep blackish-brown; palpi short, densely black scaled; antennae brown, basal joint testaceous in the centre.

Thorax brown, with narrow curved dull golden-brown scales, and black bristles; scutellum rather shiny, rich brown, with narrow curved dull-grey and brown scales, six or seven bristles to the mid lobe, and four each to the lateral lobes; metanotum deep-brown; pleurae pale ochraceous brown.

Abdomen deep-brown, with slight deep-violet reflections; narrow; border-bristles short and pale, apex testaceous, rather hairy; venter brown, hairy, testaceous at the base; the scales at the sides, in some lights under the microscope, have a dull violet-grey hue.

Legs deep-brown, with violet reflections, coxae pale ochraceous, with a number of pale hairs; venter of femora pale ochraceous, tibiae and bases of the metatarsi with a few bristles; unguis small, equal, and simple.

Wings with typical brown *Culex* scales, first submarginal cell longer and a little narrower than the second posterior cell, its stem is about one-third the length of the cell, its base nearer the base of the wing than that of the second posterior, stem of the latter about two-thirds the length of the cell; mid cross-vein long; posterior cross-vein not quite twice its own length distant from the mid.

Halteres with ochraceous stem and fuscous knob.

Length.—3.2 mm.

Habitat.—Bonny.

Time of capture.—May.

Observations.—Described from a single ♀. I do not know any species at all resembling it, yet there are no very distinctive characters. The unbanded legs and abdomen, and its general brown color, when roughly examined, make it resemble *Aedes nigra*, but it can at once be told from it by the head and wing scales, which are of typical *Culex* form.

Another ♀ differs considerably in colour, but I can detect no structural difference. It is much paler, of a general ochraceous tint, due to denudation of the scales. The thorax is paler brown with two pale median parallel stripes in front, separated by a darker line, and the scutellum has seven mid bristles, and the venter of the abdomen is paler and grey scaled. Venation, scales, unguis, etc., are similar, and it was taken in the same place and date as the type. I fancy one is full of ova, the other dark with blood.

GENUS *Panoplites*. THEOBALD (1901)

(*Mono. Culicidae*, Vol. II)

This genus differs from *Culex* chiefly in the peculiar formation of the wing scales, which are broad and asymmetrical squamae, concave at their free extremity (Fig. 13, Pl. II). This character will suffice to identify the genus. The eggs are laid singly, and taper to a point at one end. Many of the species are vicious biters, and chiefly occur along river banks. The African species here mentioned acts as the *Filaria* host.

XIX. *Panoplites africanus*. THEOBALD

(*Mono. Culicidae*, Vol. II)

Quite a number of this species occur in the collection from Asaba taken in June, July, and August. The thickly scaled wings will at once separate it from other *Culices* occurring in

the neighbourhood. The legs are broadly basally banded white, and the femora and tibiae more or less mottled; the general colour is rich brown, the abdomen being deeper brown, with apical white patches of lateral scales, and similar ochraceous basal ones. Some specimens show apical ochraceous bands; the scales are not evenly disposed and give the abdomen a slight ragged appearance. The thorax shows characteristic ornamentation under the microscope, the greater surface being covered with golden-brown scales, with lines and patches of silvery-grey scales. The specimens collected at Asaba differ in no respects from those in the other parts of West and Central Africa.

GENUS *Taeniorhynchus* ARRIBALZAGA (1891) (Modified F.V.T.)

(*Dipt. Argentina*, p. 47, and *Mono. Culicidae*, Vol. II)

Separated from *Culex* by Arribalzaga chiefly on account of the palpal structure and unguis and banded rostrum. His genus, however, contains three totally diverse species. I have, therefore, remodelled it upon his *T. fasciolatus* (Vide *Mono. Culicidae*).

The only feature I need point out here is that the wings are always covered along the veins with thick elongated scales, giving the wings a densely scaled appearance, but quite different to *Panoplites* in form. I know nothing of the life-history of any of the species in this genus.

XX. *Taeniorhynchus aurites*. THEOBALD

(*Mono. Culicidae*, Vol. II)

Eight or nine ♀'s of this pretty golden-yellow gnat were taken at Bonny and Ogugumanga. One bears on the label 'Taken in the bush opposite St. Stephen's Cathedral.' They were captured in May, June, and July. It can be told from the other yellow African mosquito by the thorax being honey-yellow and unadorned; the hind legs have apical dark bands to the metatarsi and tarsi, and the wings brilliant orange-yellow.

XXI. *Taeniorhynchus annettii*. THEOBALD

(*Mono. Culicidae*, Vol. II)

A ♂ and eight ♀'s taken at Old Calabar at the Vice-Consulate in April, and at Bonny.

It resembles *T. aurites* but the sixth vein is dark scaled; there is darker thoracic ornamentation and apical dark banding to the fore and mid legs, more or less distinct; the abdomen has apical deep-violet bands.

GENUS *Aedes*. MEIGEN (1818)

(*Syst. Besch.* Vol. I, p. 13, 1818)

Palpi short in both ♂ and ♀. Head clothed with both flat and narrow-curved scales, the flat scales predominating; scutellum with narrow-curved scales only. Fork-cells of the wings moderately long; scales on the wings very similar to *Culex*, there being always long, thin, lateral scales to the veins, which are not seen in other genera of the *Aedeomyia*.

Two species occur in the genus in Africa.

XXII. *Aedes nigra*. THEOBALD

(Mono. Culicidae, Vol. II)

Five ♀'s and one ♂ of this small dark *Aedes* only about 2mm. long. Taken at Old Calabar in April. It can readily be told by its black appearance, unbanded legs, abdomen, and absence of thoracic ornamentation. From the *Uranotaenia* it can at once be distinguished by the relative greater length of the fork-cells.

GENUS *Uranotaenia*. ARRIBALZAGA (1891)

(Dipt. Argentina, p. 63, 1891)

Palpi short in the ♂ and ♀ as in *Aedes*, but the fork-cells are very small, especially the first submarginal fork-cell. There are always flat scales, usually brilliant in places on the mesonotum and on the scutellum, and the head is entirely covered with flat scales. Many of the species bite severely. The larvae are often brilliantly coloured with red, blue, and green, and seem to be intermediate between *Anopheles* and *Culex* in structure.

XXIII. *Uranotaenia domestica*. THEOBALD

(Mono. Culicidae, Vol. II)

Two specimens of this beautiful *Uranotaenia* taken at Old Calabar at the Vice-Consulate, in April. One badly damaged.

It can easily be identified by the bright, chestnut-brown thorax, with a small, silvery spot on each side in front, another on the roots of the wings, a bright, silver-scaled scutellum; the abdomen is almost black, with white lateral spots, and the legs are black with a white spot at the apex of the tibiae and femora, and a silvery band near the apex of the hind femora.

Length.—4 mm.

XXIV. *Uranotaenia annulata*. THEOBALD

(Mono. Culicidae Vol. II)

Three ♀'s and three ♂'s taken at Bonny in May. A very marked little *Uranotaenia*, with chestnut-brown mesothorax and sharply contrasted pale creamy pleurae and head, the latter having a dark median line. The abdomen is brown, and has apical grey or white bands. Legs brown; the hind ones with the metatarsi and first two tarsi with apical white bands, and the last two joints pure white.

XXV. *Uranotaenia caeruleocephala*. THEOBALD

(Mono. Culicidae, Vol. II)

Eight ♀'s taken in April at Old Calabar. It is a beautiful little deep-brown species, easily identified by its sky-blue head. The legs and abdomen are unbanded. On the thorax may be seen a line of white scales at the sides, just in front of the wings.

Length.—2.5 mm.

PLATES TO APPENDIX

PLATE I

- FIG. 1. *Eretmapodites quinquevittata*.—Fore and mid ungues of ♂, and fore ungues of ♀ :
♂ palpus and apex of ♂ hind legs.
- FIG. 2. *Stegomyia irritans*. Nov. Sp.—♂ palpus and cephalic ornamentation.
- FIG. 3. *Stegomyia nigricephala*. Nov. sp.—*a*, wing of ♀ ; *b*, head ; *c*, abdominal ornamentation ; *d*, fore ungues of ♀ .
- FIG. 4. *Culex duttoni*. Nov. Sp.—*a*, thorax of ♀ ; *b*, markings on denuded thorax ; *c*, ♂ palpus ; *d*, abdominal ornamentation.
- FIG. 5. *Culex decens*. Nov. sp.—♂ palpus.
- FIG. 6. *Culex pruina*. Nov. sp.—♂ and ♀ abdominal ornamentation.

PLATE II

- FIG. 7. *Culex pruina*. Nov. sp.—*a*, wing of ♀ ; *b*, fore ungues of ♂
- FIG. 8. *Culex invenustus*. Nov. sp.—*a*, wing of ♀ ; *b*, head ornamentation.
- FIG. 9. *Culex invenustus*. Nov. sp.—Fore leg to first tarsal joint.
- FIG. 10. *Culex nebulosus*. Nov. sp.—*a*, wing of ♂ ; *b*, cephalic ornamentation ; *c*, abdominal ornamentation.
- FIG. 11. *Culex rima*. Nov. sp.—*a*, wing of ♀ ; *a*¹, apical wing scales ; *a*², basal scales ; *b*, clypeus.
- FIG. 12. *Culex invidiosus*. Nov. sp.—*a*, scutellar bristles ; *b*, wing of ♀
- FIG. 13. Wing scales of *Panoplites*.

PLATE III

- FIG. 14. *Culex metallicus*. THEOBALD.—*a*, thoracic ornamentation ; *a*¹ and *a*² enlarged scales ; *b*, ♂ palpus ; *c*, fore and hind ♀ ungues ; *d*, apex of antenna ; *e*, wing fringe ; *f*, ♂ genitalia ; *g*, wing scales ; *i*, another form of wing scales ; *h*, fore and hind ♂ ungues.

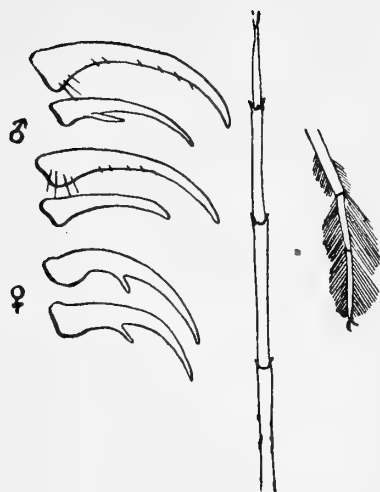


FIG. 1

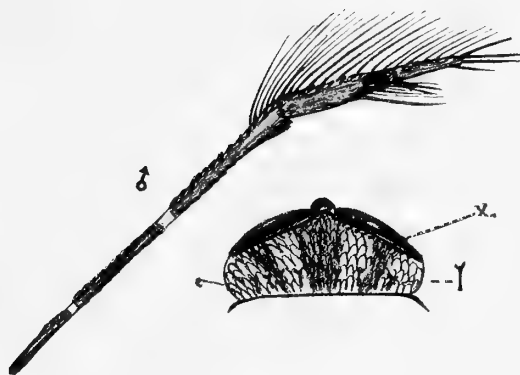


FIG. 2

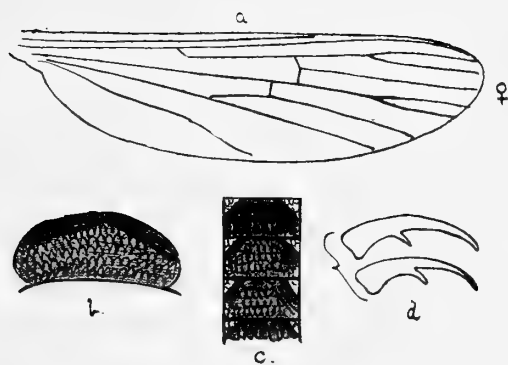


FIG. 3

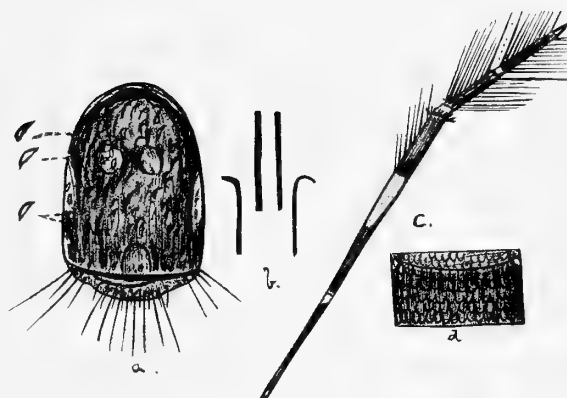


FIG. 4



FIG. 5

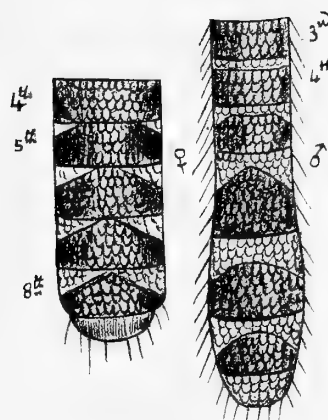
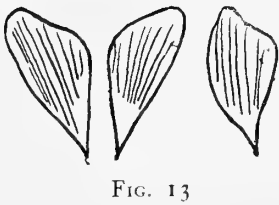
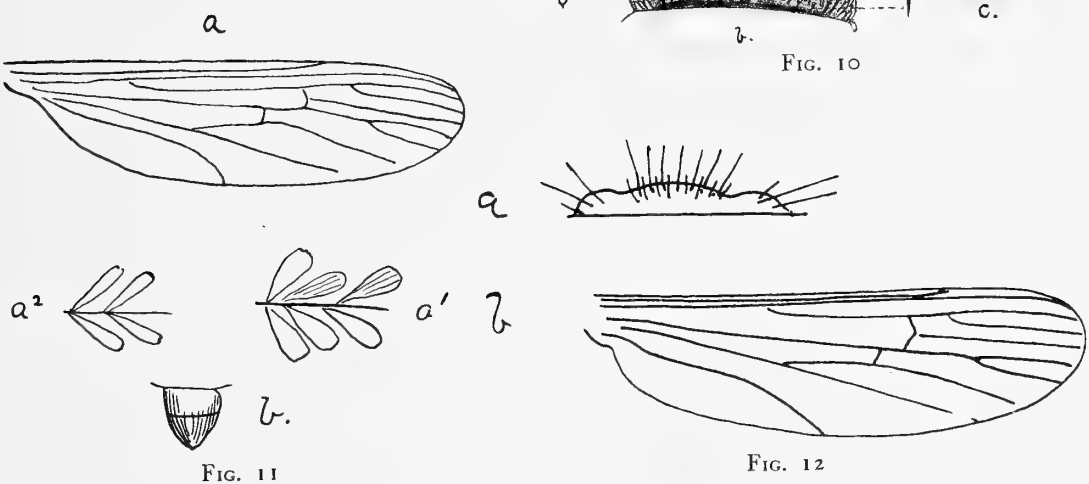
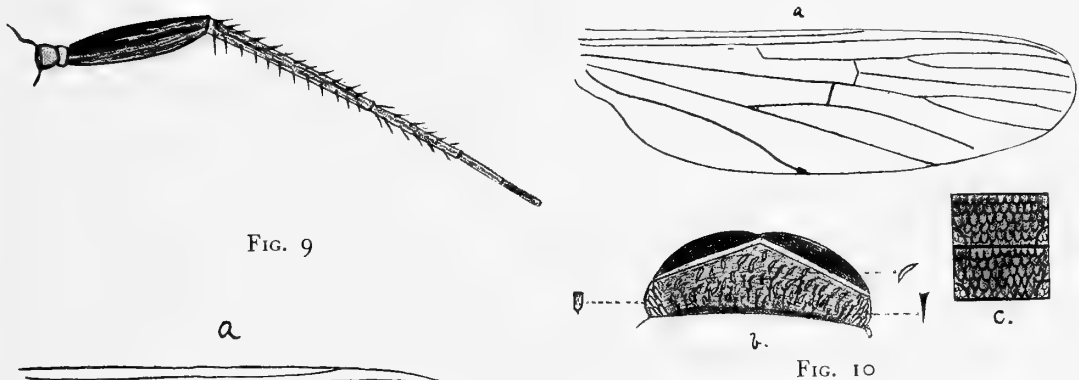
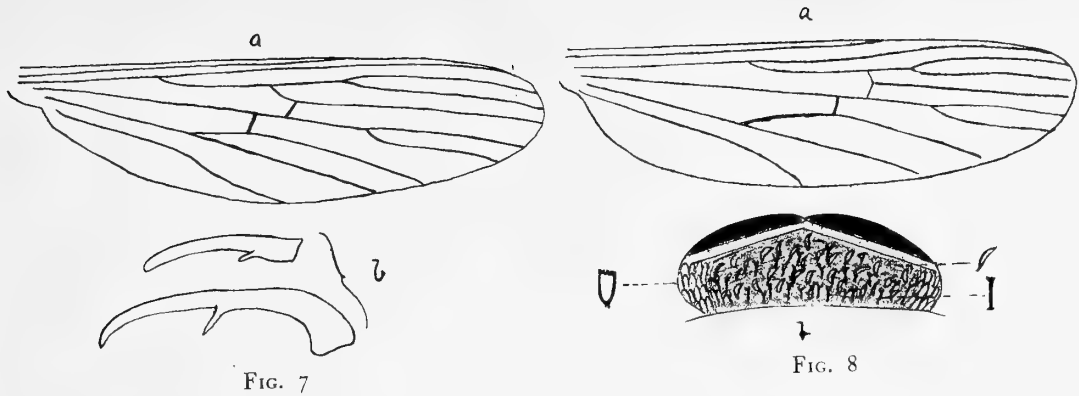


FIG. 6



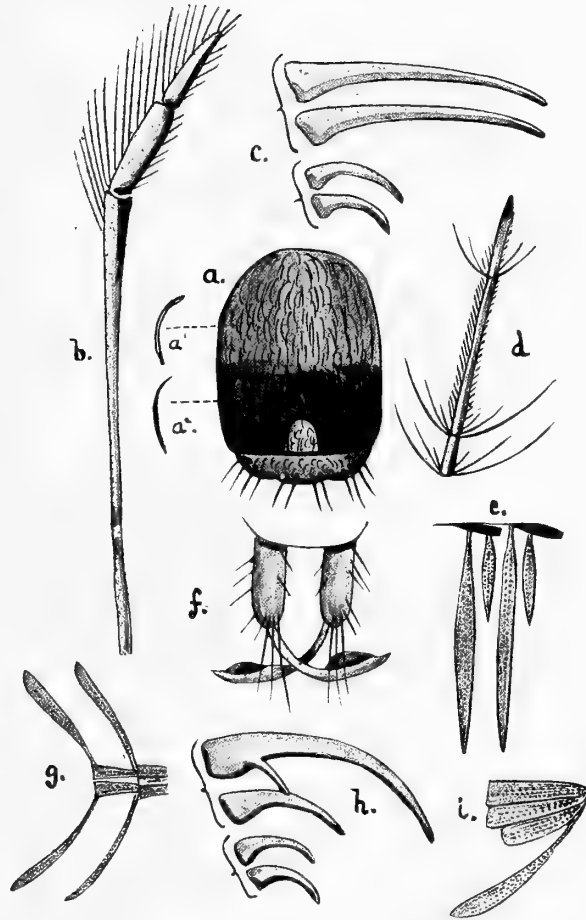


FIG. 14

DESCRIPTION OF PLATES

PLATE I

- FIG. 1. *Filaria cypseli*. Nov. sp. ♂ and ♀ : natural size.
- FIG. 2. *F. cypseli*. Nov. sp. Head end of ♀. *a*, alimentary tract; *b*, oesophagus; *c*, opposite vaginal orifice; *d*, uterus full of ova; *e*, vagina. The nerve collar is indicated by the dark band across the anterior portion of the oesophagus.
- FIG. 3. *F. cypseli*. Tail end of ♀. *a*, position of anus; *b*, uterus.
- FIG. 4. *F. cypseli*. Head end of ♂. *a*, oesophagus; *b*, intestine.
- FIG. 5. *F. cypseli*. Tail end of ♂. The worm has been ruptured near the extreme end, where the body contents are extruded. *a*, alimentary tract; *b*, opposite position of anal orifice; *c*, spermatic tube; *d*, spicules.

PLATE II

- FIG. 1. *Filaria spiralis avium*. Nov. sp. ♂ and ♀ : natural size.
- FIG. 2. *F. spiralis avium*. Anterior end of ♀, and portion of first spiral. *a*, alimentary tract; *b*, oesophagus; *c*, opposite the position of vaginal orifice; *d*, uterus; *e*, vagina.
- FIG. 3. *F. spiralis avium*. Posterior end of ♀, and portion of last spiral. *a*, alimentary canal; *b*, opposite the anal orifice, which is seen to be surrounded by five delicate lips, giving a rosette appearance; *c*, the lateral ridge as seen in the concavities of the coils; *d*, uterus, full of ova.
- FIG. 4. *F. spiralis avium*. Posterior end of the ♀, side view; *a*, alimentary canal; *b*, opposite the position of anal orifice: seen here as a baying in the cuticle; *c*, the lateral cuticular ridge as seen on the convexities of the spirals; *d*, uterus.
- FIG. 5. *F. spiralis avium*. The embryo: stained specimen of the blood ($\times 250$). The dark spots round the worm are the nuclei of the red corpuscles. The sheath of the worm is distinctly shewn both at the head and tail ends of the worm.

PLATE III

- FIG. 1. *Filaria spiralis avium*. Tail end of ♂ shewing its shape; *a*, opposite the position of the anal orifice; *b*, alimentary tract; *c*, spermatic tube.
- FIG. 2. *F. spiralis avium*. Shews the spicular arrangement of the male. The two spicules are seen extruded through the wide crater-like anal orifice, situated on a low papilla. Behind is indicated the lateral cuticular flange which here comes to the ventral surface, to form with the one of the other side a sort of hollow cone at the bottom of which is the anal orifice.

PLATE IV

- FIG. 1. *Filaria fusiformis avium*. Nov. sp. ♂ and ♀ : natural size.
- FIG. 2. *F. fusiformis avium*. Anterior end of ♀ shewing its shape, and *a*, the position of the vaginal orifice.

- FIG. 3. *F. fusiformis avium*. Posterior end of ♀ shewing its shape; *a*, alimentary tube; *b*, near the termination of the uterine tube.
- FIG. 4. *F. fusiformis avium*. The embryos: a specimen of stained blood shewing the embryos inside, partly and completely out of their sheaths, also an empty sheath. The position of some of the 'spots' is also seen ($\times 350$).

PLATE V

- FIG. 1. *Filaria spiralis major avium*. Nov. sp. ♀ and ♂: natural size.
- FIG. 2. *F. spiralis major avium*. Anterior end of ♀. *a*, opposite the anal orifice; *b*, uterus; *c*, vagina.
- FIG. 3. *F. spiralis major avium*. Tail end of ♀. *a*, opposite the anal orifice. The cuticular knobs are well seen on the convexities of the spirals.
- FIG. 4. *F. spiralis major avium*. Anterior end of ♂. *a*, oesophagus; *b*, alimentary canal; *c*, spermatic tube.
- FIG. 5. *F. spiralis major avium*. The strongly incurved tail of the ♂. *a*, opposite the position of the anal orifice and spicules.

PLATE VI

- FIG. 1. *Filaria spiralis major avium*. The embryo in a specimen of stained blood, shews the position of some of the 'spots' and the characteristic wire nail shaped posterior end ($\times 250$).
- FIG. 2. *F. shekletonii*. Nov. sp. The embryo in a specimen of stained blood. The position and characters of the 'spots' are well marked, as well as the sharply pointed tail ($\times 250$).
- FIG. 3. *F. shekletonii*. ♀: natural size.
- FIG. 4. *F. shekletonii*. Head end of ♀. *a*, oesophagus; *b*, the alimentary tract; *c*, opposite vaginal orifice; *d*, uterus.
- FIG. 5. *F. shekletonii*. The posterior end of ♀. *a*, intestine; *b*, opposite anal orifice; *c*, uterus.

PLATE VII

- FIG. 1. *Filaria falciformis*. Nov. sp. ♂ and ♀: natural size.
- FIG. 2. *F. falciformis*. Nov. sp. Head end of ♀. *a*, oesophagus; *b*, intestinal canal; *c*, opposite vaginal orifice; *d*, vagina; *e*, uterus.
- FIG. 3. *F. falciformis*. Tail end of ♀. *a*, position of anal orifice; *b*, ovary; *c*, uterus; *d*, the corrugated cuticle.
- FIG. 4. *F. falciformis*. Head end of ♂. *a*, oesophagus; *b*, intestine; *c*, opposite position of nerve collar crossing the oesophagus.
- FIG. 5. *F. falciformis*. Tail end of ♂ shewing the spicules *a* and *b* extruded through the wide anal orifice; *c*, papillae; *d*, base of spicules.

PLATE VIII

- FIG. 1. *Filaria falciformis*. Tail end of ♂ shewing spicular arrangement not extruded; numerous spermatozoa are seen.
- FIG. 2. *F. falciformis*. The embryo in a specimen of stained blood; shews the characteristic 'spot' ($\times 250$).

FIG. 3. *F. bibulbosa*. Nov. sp. ♂ and ♀ : natural size.

FIG. 4. *F. bibulbosa*. Head end of ♀. *a*, intestinal tract; *b*, oesophagus; *c*, opposite vaginal orifice; *d*, uterus; *e*, vagina.

FIG. 5. *F. bibulbosa*. Tail end of ♀; *a*, opposite anal orifice; *b*, intestine; *c*, distal end of ovary.

PLATE IX

FIG. 1. *Filaria bibulbosa*. Head end of ♂; *a*, intestinal canal; *b*, oesophagus; *c*, spermatic tube.

FIG. 2. *F. bibulbosa*. Tail end of ♂; note the single extruded spicule.

FIG. 3. *F. bibulbosa*. The embryos in stained blood. Specimen shewing their comma-shape and 'spots' ($\times 250$).

PLATE X

FIG. 1. *Filaria capsulata*. Nov. sp. ♂ and ♀ and cyst containing worms : natural size.

FIG. 2. *F. capsulata*. The cyst with ♀ worm enclosed were highly magnified. The ♂ had been removed.

FIG. 3. *F. capsulata*. Head end and portion of the body of ♀; *a*, oesophagus; *b*, intestine; *c*, opposite vaginal orifice; *d*, vagina; *e*, uterine horn.

FIG. 4. *F. capsulata*. Tail end of ♀; *a*, intestine; *b*, opposite anal orifice.

FIG. 5. *F. capsulata*. The embryo in stained blood preparation ($\times 250$).

PLATE XI

FIG. 1. *Filaria capsulata*. The ♂ complete. *a*, oesophagus; *b*, intestine; *c*, spermatic tube; *d*, tail end; *e*, head end.

FIG. 2. *F. capsulata*. Tail end of ♂. *a*, opposite anus and single partly extruded spicule; *b*, intestine; *c*, head end of ♂; *d*, head end of ♀.

FIG. 3. *F. phoenicopteri*. Nov. sp. ♂ : natural size.

FIG. 4. *F. phoenicopteri*. Head end of ♂. *a*, oesophagus; *b*, opposite oral orifice.

FIG. 5. *F. phoenicopteri*. Tail end of ♂ shewing single spicule extruded. *a*, intestine.

PLATE XII

FIG. 1. *Filaria serpentiformis*. Nov. sp. The embryo in stained blood preparation ($\times 250$).

FIG. 2. *F. opobensis*. Nov. sp. The embryo in stained blood preparation ($\times 350$).

FIG. 3. *F. calabarensis*. Nov. sp. The embryo in stained blood preparation ($\times 250$).

PLATE XIII

FIG. 1. *Filaria cypseli*. Embryo with sheath—fresh specimen ($\times 550$).

FIG. 2. *F. spiralis avium*. Embryo with sheath—fresh specimen ($\times 550$).

FIG. 3. *F. fusiformis avium*. Embryo, and its head ending showing prepuce, papilla, and spine protruded and retracted ($\times 550$).

FIG. 4. *F. spiralis major*. Embryo and sheath ($\times 550$).

FIG. 5. *F. falciformis*. Embryo ($\times 550$).

FIG. 6. *F. bibulbosa*. Embryo ($\times 550$).

PLATE XIV

FIG. 7. *Filaria capsulata*. Embryo ($\times 550$).

FIG. 8. *F. serpentiformis*. Embryo ($\times 550$).

PLATE XV

- FIG. 1. Transverse section of proboscis of the female *Anopheles costalis* near its tip ($\times 460$). *lr-ep*, labrum-epipharynx; the two portions are shewn separated by a thin red transverse band; *h*, hypopharynx, with salivary canal at its centre; *m*, mandible; *mx*, maxilla; *lb*, labella; *t*, tip of labium; *fh*, superior region of inner surface of labella from which arises a feltwork of fine hairs; *ch*, inferior region of inner surface from which coarse hairs arise; *r*, a ridge of thickened chitin on the middle region of the inner surface, which above at its base enters into the articulation of the labella and labium.
- FIG. 2. Transverse section of proboscis at the level of the labella joints ($\times 460$). *lr-ep*, *h*, *m*, *mx*, as in fig. 1; *mx.p*, maxillary palp; *l*, lateral pear-shaped area at extremity of labella; *ln*, nerve to the labella; *a*, chitinous articulating surface of the labium; *k*, triangular area, occupied by a loose delicate membrane hanging from beneath the portion of the upper chitinous surface of the labium, which is prolonged to the extreme tip of the proboscis. In the section, the cut edge of the membrane is shewn as an irregular line. In this figure the labrum is not represented.
- FIG. 3. Transverse section about the level of the middle of the proboscis ($\times 460$). *lr-ep*, *h*, *m*, *mx*, *mx.p* as in figs. 1 and 2; *l*, labium; *ltr*, trachea to the labium; *ln*, nerve to the labium; *r*, lateral chitinous ridge of the labium; *lm*, labellar muscles. In this figure the labrum is not represented.

PLATE XVI

- FIG. 1. Transverse section at the base of the proboscis of the female *Anopheles costalis* ($\times 460$). *lr*, labrum; *ep*, epipharynx; *ep.r*, lateral supporting chitinous ridge of the epipharynx containing core of chitin forming cells; *h*, hypopharynx with salivary gutter; *m*, mandible; *mx*, maxilla; *mx.p*, maxillary palp; *o'*, a concave region on the inner surface of the maxillary palp, against which the mandible fits, indicating the relation of its origin; *o''*, a similar region for the maxilla; *p.m*, muscle of the maxillary palp; *l*, labium, note the shape at this level as compared with sections, plate xv, fig. 3; *r*, lateral chitinous ridge of labium; *ltr*, labial trachea; *ln*, labial nerve.
- FIG. 2. Transverse section of proboscis just before the separation of the various mouth parts from each other ($\times 400$). *c*, clypeus; *f*, upper posterior angle of the fulcrum; *lr*, proximal extremity of labrum, note the cubical cells; *lr.p*, chitinous prolongation of the labium within the clypeus; *em*, epipharyngeal muscle; *ep*, epipharynx; *h*, hypopharynx, the apex of the salivary receptacle is seen below, supported by two lateral chitinous bars; *m*, mandible; *mx*, maxilla, note its sickle shape; *mx.p*, maxillary palp; *pn*, nerve to maxillary palp; *pm*, muscle to the maxillary palp; *l*, labium; *ln*, labial nerve; *ltr*, labial trachea; note the line of cleavage of the labium from the other mouth parts.

PLATE XVII

- FIG. 1. Transverse section of the head of *Anopheles costalis*, at the level of the middle of the ascending portion of the pharynx ($\times 360$). *p*, ascending portion of the pharynx; *pd*, middle membranous portion of the upper wall of the pharynx, consisting of a layer of low cubical epithelium; *pv*, lower chitinous plate of the pharynx; *pm*, pharyngeal muscle; *lbr.m*, fan-shaped labral muscle; *sd*, common salivary duct;

rm, muscle to the salivary receptacle; *mx.p'*, intercranial maxillary process; *zm*, muscle attaching maxillary process to the occipital region of head; *lm'*, muscle to base of labium; *ln*, nerve to the proboscis; *ltr*, trachea to the proboscis; *mm*, muscle to the base of the mandible.

PLATE XVIII

FIG. 1. Semi-diagrammatic sagittal section through the head and proboscis of the female *Anopheles costalis* ($\times 200$). *lbr*, labrum; *ep*, epipharynx; *h*, hypopharynx; *l*, labium; *p'* ascending portion to the pharynx; *p''*, horizontal portion; *n*, nerve to the proboscis; *oe*, oesophagus; *tr*, trachea to the proboscis; *s.r*, salivary receptacle; *s.d*, common salivary duct; *f.m*, muscle to the salivary receptacle; *x*, chitinous ridge or under surface of ventral wall of the first part of the pharynx, from which (*f.m*) the muscle to the salivary receptacle arises; *c*, clypeus; *p.m*, pharyngeal muscle; *lbr.m*, labral muscle inserted into the prolongation of the labrum; *e.m*, epipharyngeal muscle arising from the fulcrum; *a*, commencement of the labrum: below this on the upper wall of the pharynx are the 'taste papillae'; *f*, fulcrum; *s.o.g*, supra-oesophageal ganglion; *i.o.g*, infra-oesophageal ganglion; *s.o*, specialized hairs; *mx.p*, intercranial maxillary process; *v.c*, ventral commissure; *d.v*, dorsal vessel; *d.t*, main trachea to the head; *e*, eye.

FIG. 2. Transverse section at the level of the junction of the first and second part of the pharynx shewing the group of specialized hairs ($\times 530$). *p*, pharynx; *z*, that part of the exoskeleton which is folded in beneath the eyes.

PLATE XIX

FIG. 1. Semi-diagrammatic longitudinal horizontal section of the head and proboscis of the female *Anopheles costalis* ($\times 180$). *bm*, muscle to the pumping organ, the middle membranous portion of the pharynx; *zm*, muscle attaching the maxillary process to the occipital region of the skull; *ltr*, trachea and nerve to the proboscis; *lm'*, muscle to the base of the labium, arising from the under surface of the maxillary process; *rm*, muscle to the salivary receptacle; *sr*, salivary receptacle and duct; *s*, V-shaped opening of salivary receptacle; *h*, hypopharynx, the salivary gutter runs along its centre; *mx.p*, maxillary palp; *lm*, origin of labellar muscle; *mx.p'*, intercranial process of the maxilla; *e*, eye.

FIG. 2. Section of the distal end of the labium and labellae ($\times 410$). *lm*, labellar muscle; *lm'*, longitudinal tendon of labellar muscle; *r*, lateral chitinous ridge of the labium, from which the labellar muscles arises; *a*, chitinous process at the base of the labella into which the long tendon of the labellar muscle is inserted; *lb*, labella; *ln*, the termination of the labellar nerve; *g*, ganglionic structure in the interior of the labella, shewing the fibres of the labellar nerve ramifying over its surface; *ch*, coarse hairs projecting downwards between the labellae and arising from their inner surface.

FIG. 3. Drawing of a cleared specimen of the distal end of the labium and of the labellae of the female *Anopheles costalis* ($\times 390$). *lb*, labella; *l*, labium; *g*, groove on the upper surface of the labium in which the stylets are enclosed; *a*, the labellae articulation, observe the angle for the insertion of the long tendon of the labellar muscle; *r*, lateral chitinous ridge of labium; *tl*, tip of the labium.

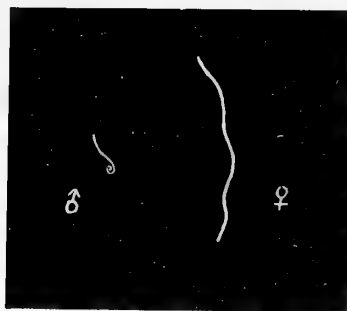


FIG. 1

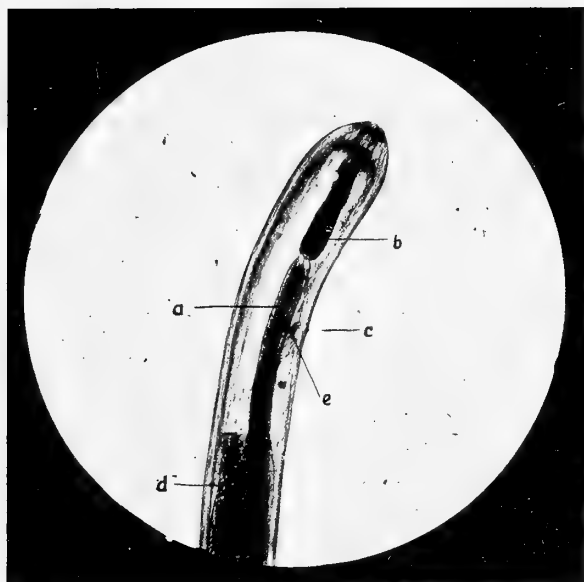


FIG. 2

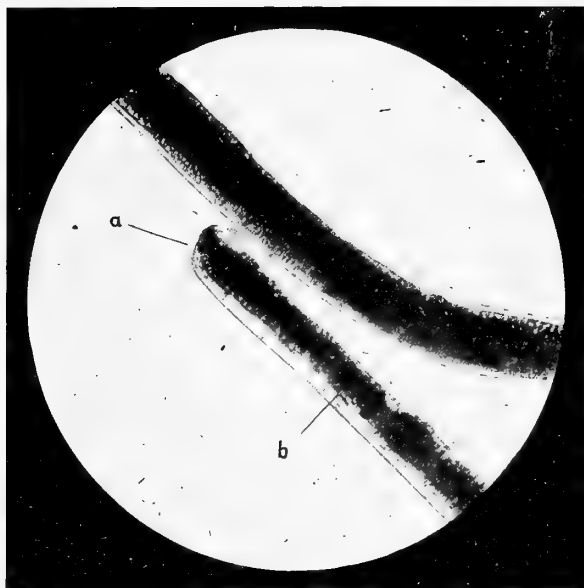


FIG. 3

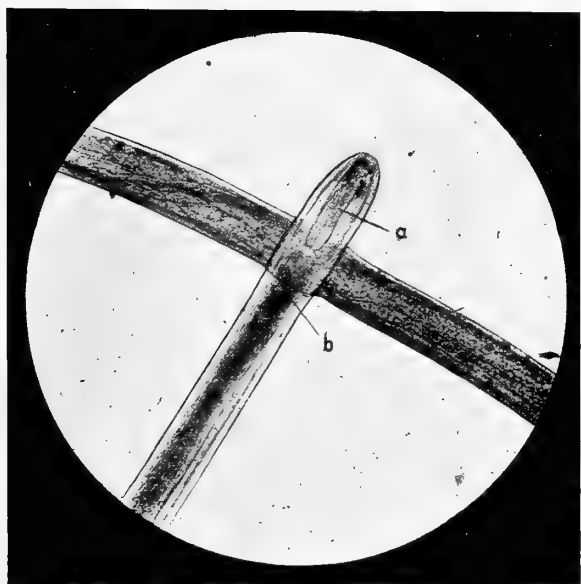


FIG. 4

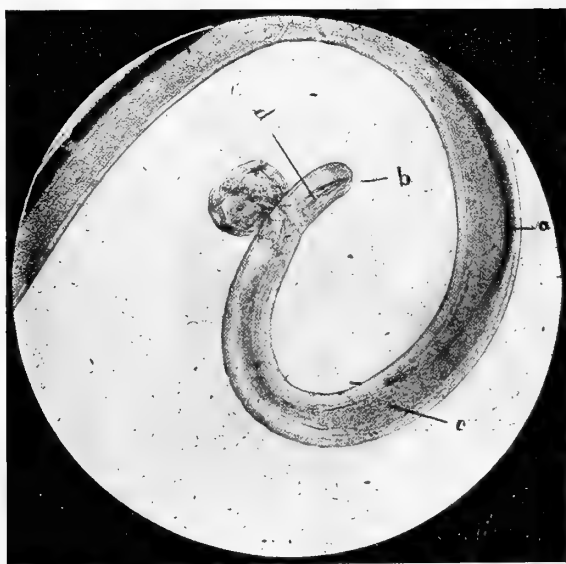


FIG. 5

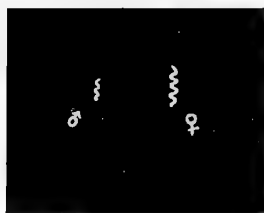


FIG. 1

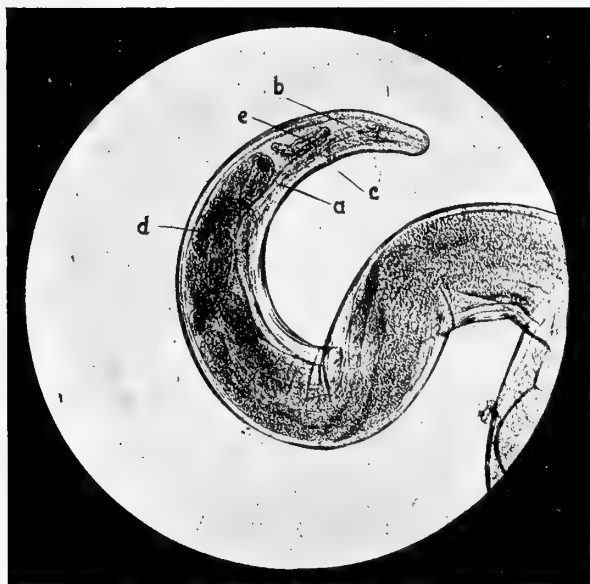


FIG. 2

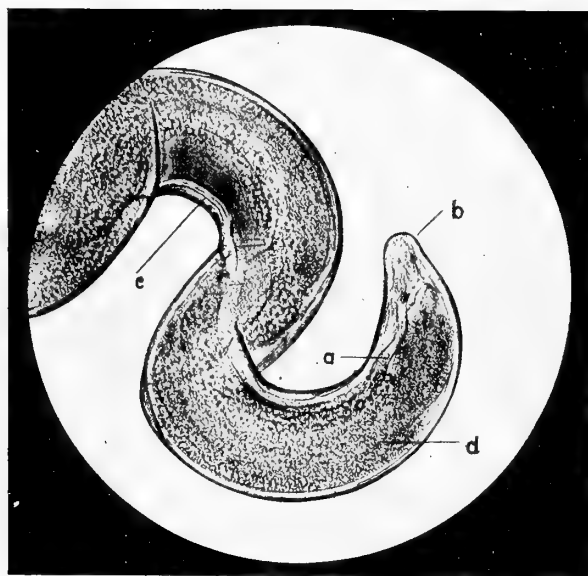


FIG. 3

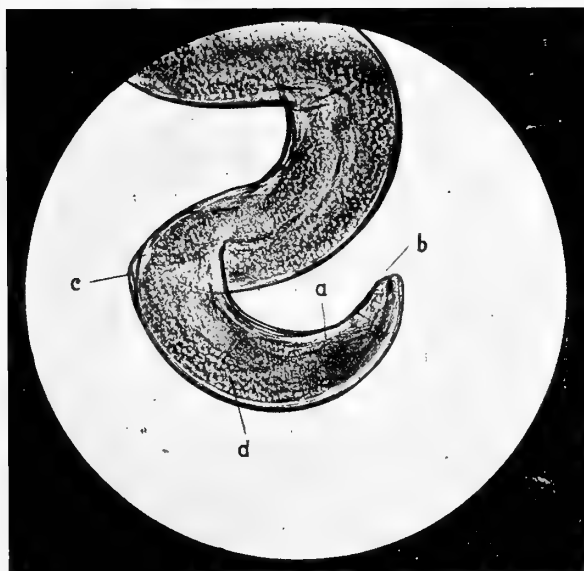


FIG. 4

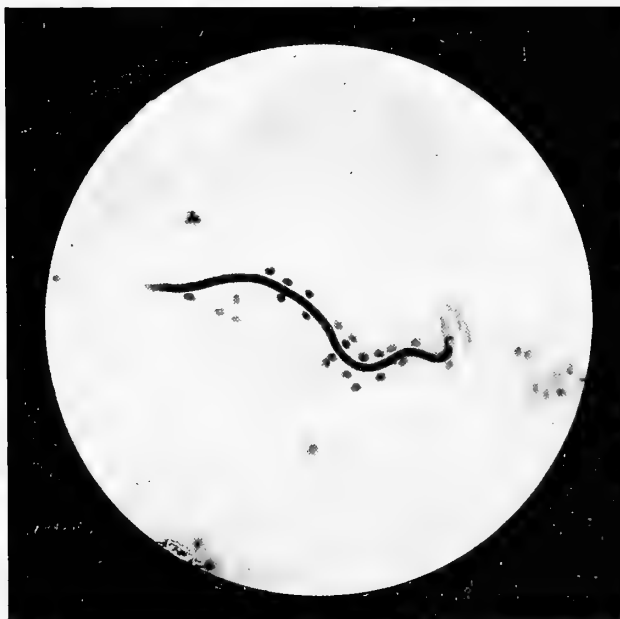


FIG. 5



FIG. 1



FIG. 2

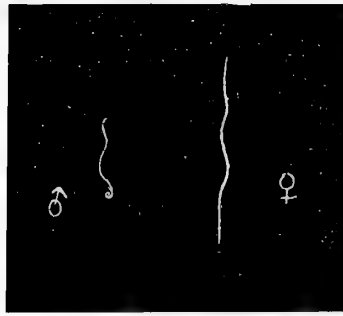


FIG. 1

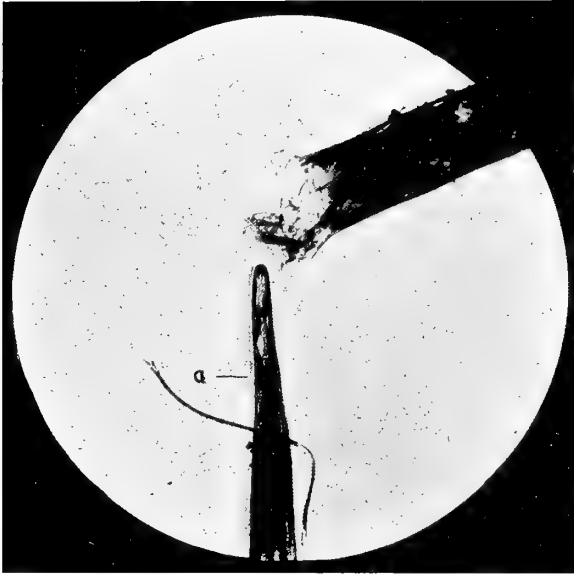


FIG. 2

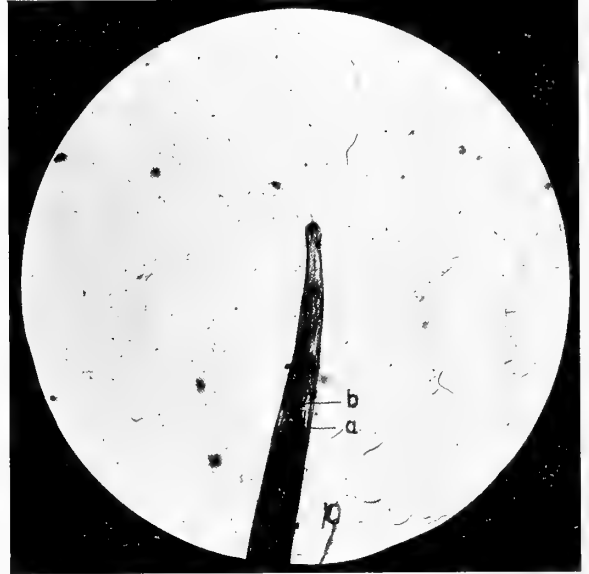


FIG 3



FIG. 4

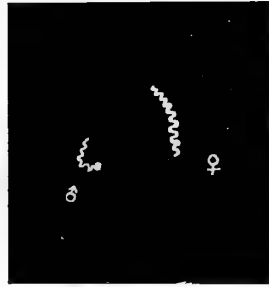


FIG. 1

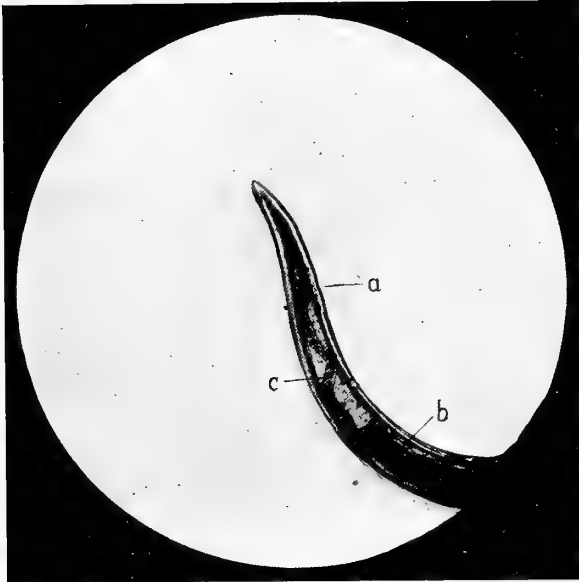


FIG. 2



FIG. 3

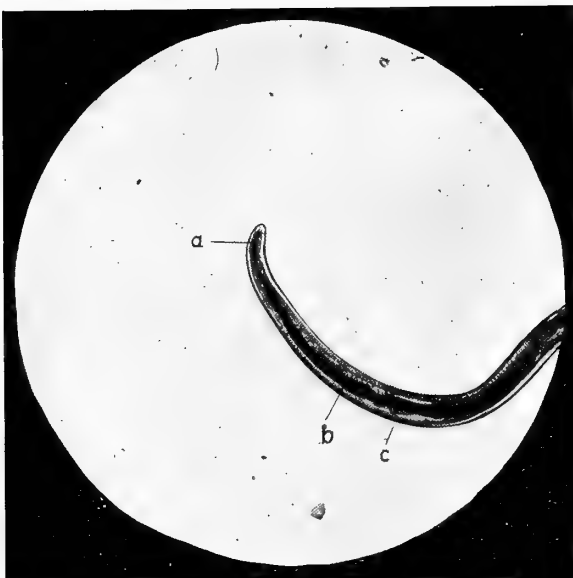


FIG. 4



FIG. 5

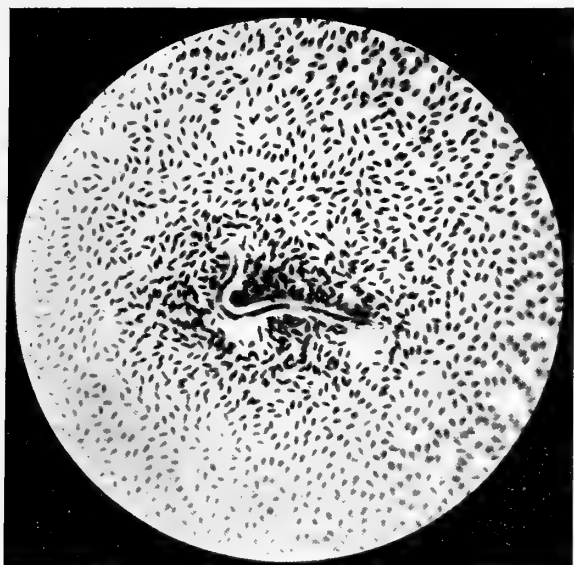


FIG. 1

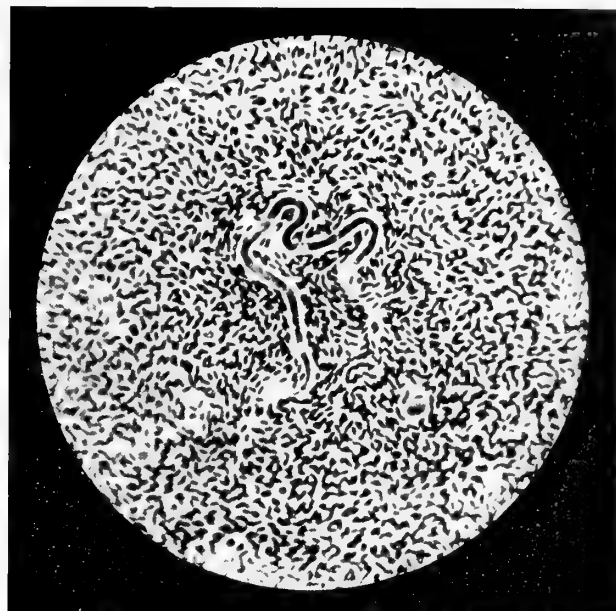


FIG. 2



FIG. 3

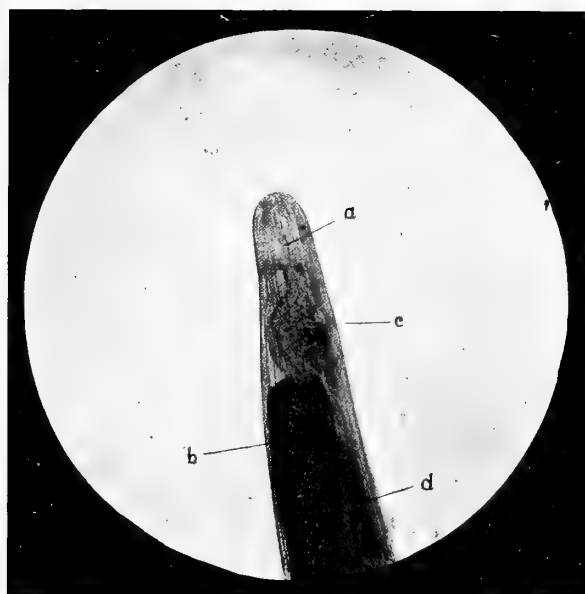


FIG. 4

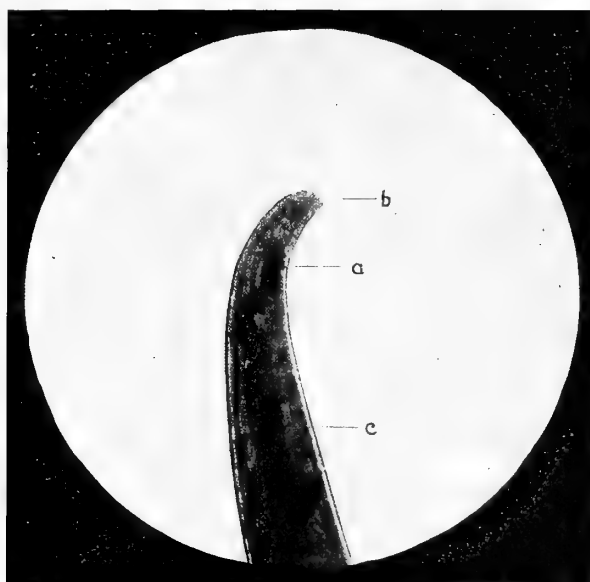


FIG. 5

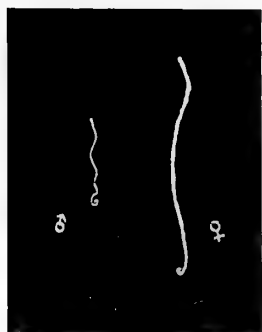


FIG. 1

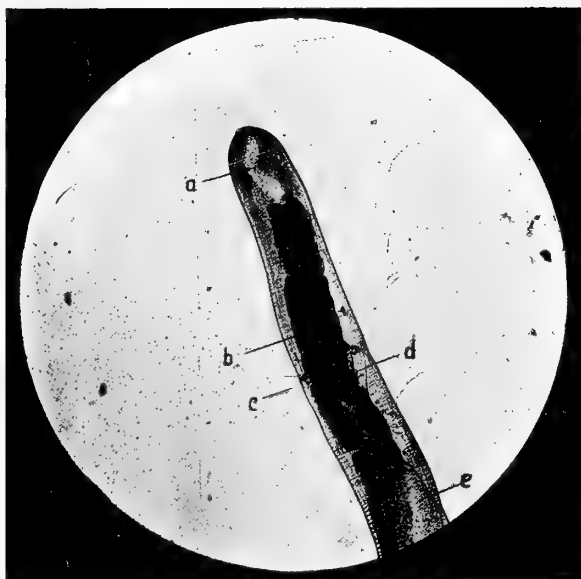


FIG. 2

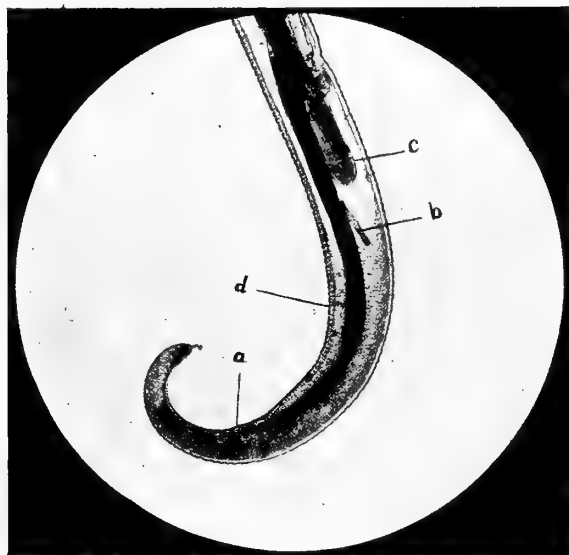


FIG. 3

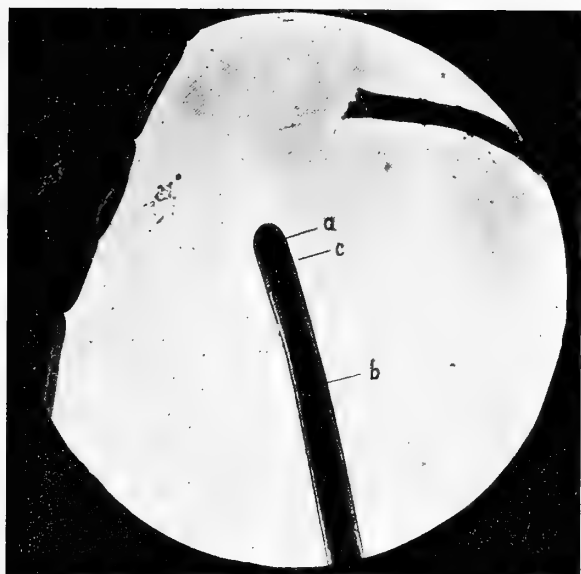


FIG. 4

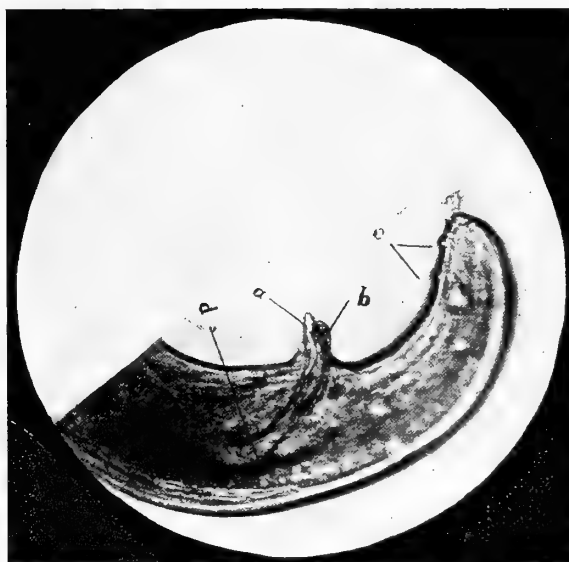


FIG. 5

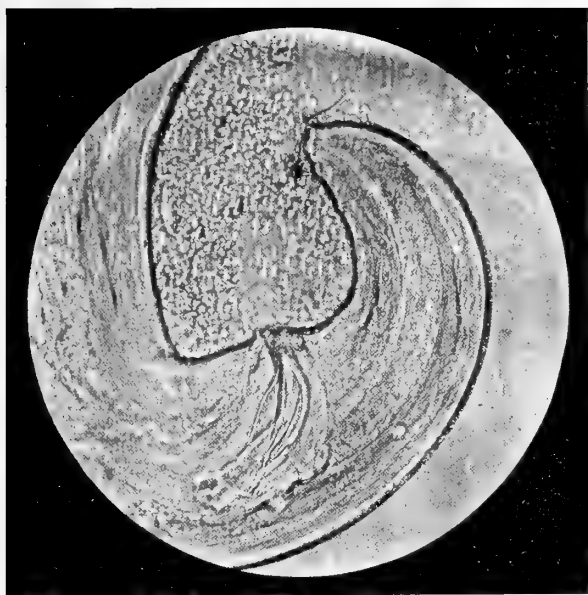


FIG. 1

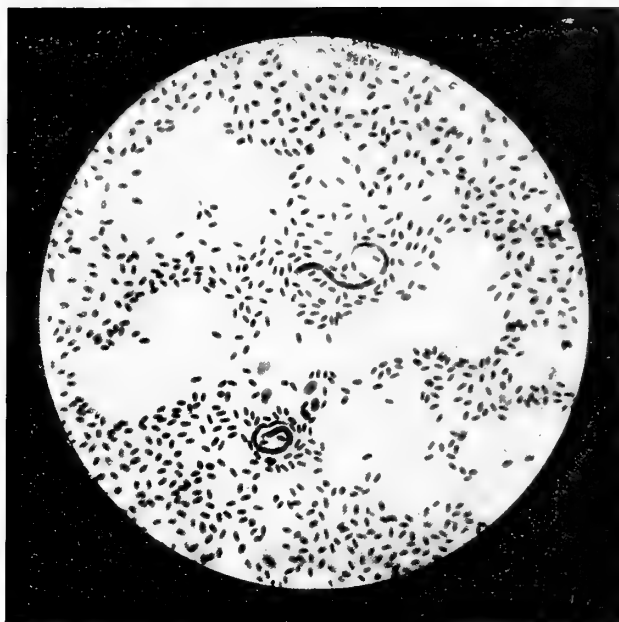


FIG. 2

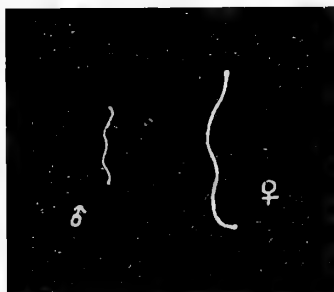


FIG. 3

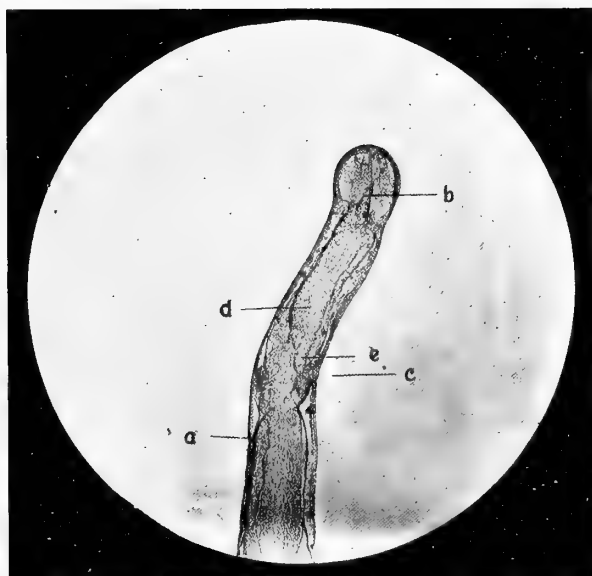


FIG. 4

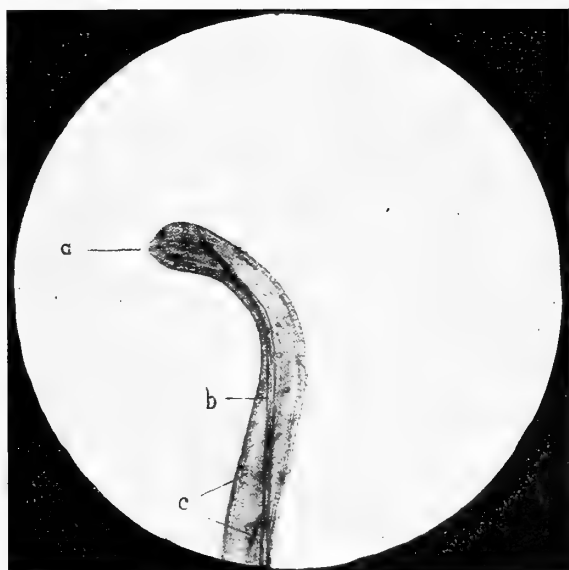


FIG. 5

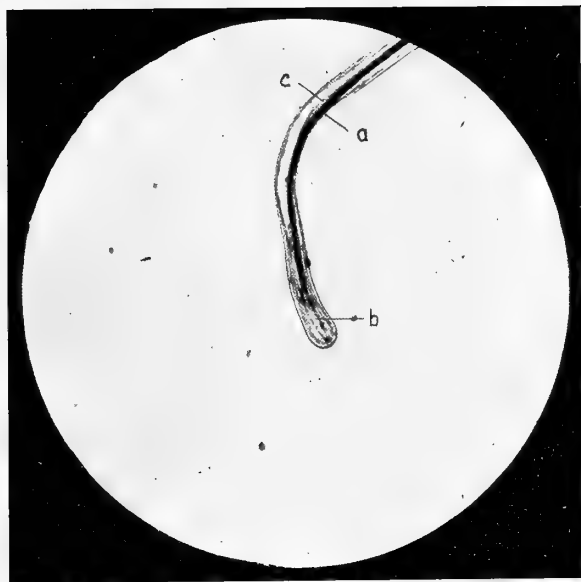


FIG. 1



FIG. 2

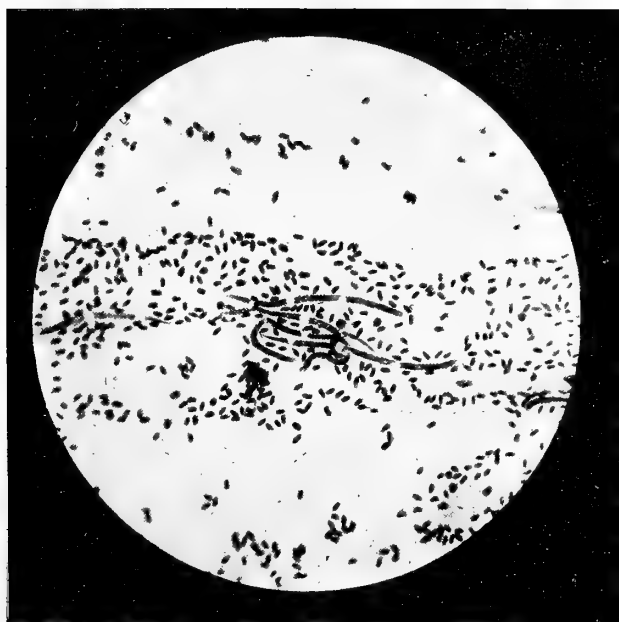


FIG. 3

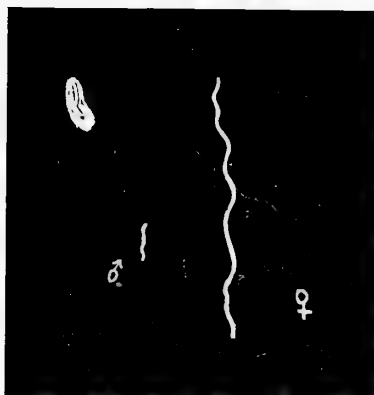


FIG. 1



FIG. 2

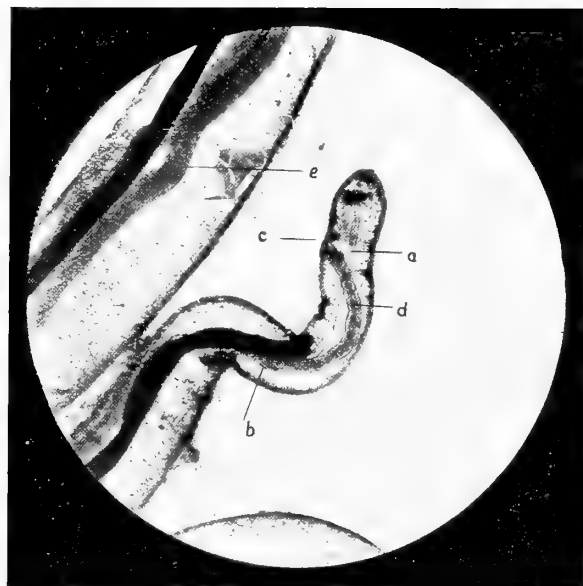


FIG. 3



FIG. 4

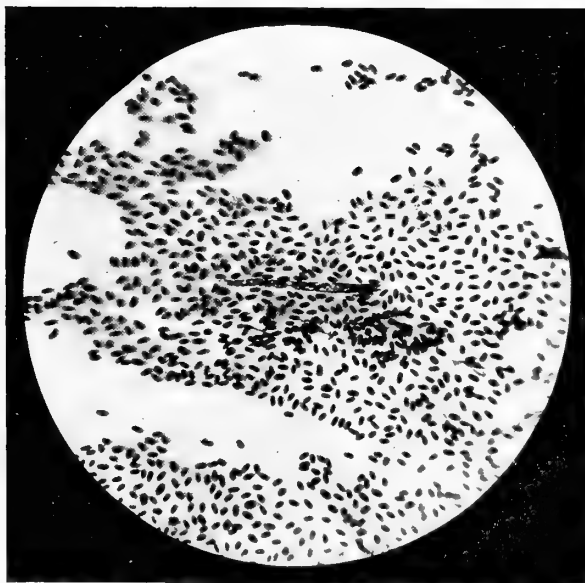


FIG. 5

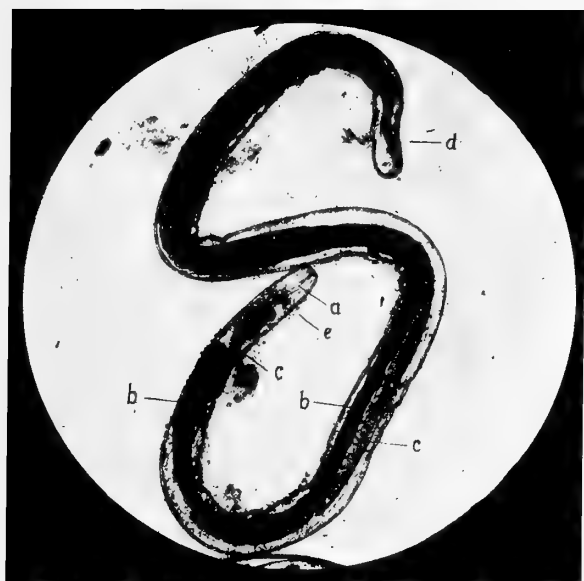


FIG. 1



FIG. 2



FIG. 3



FIG. 4

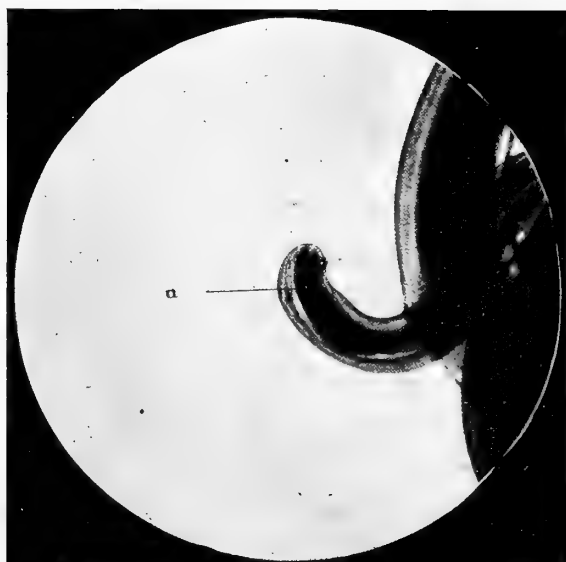


FIG. 5

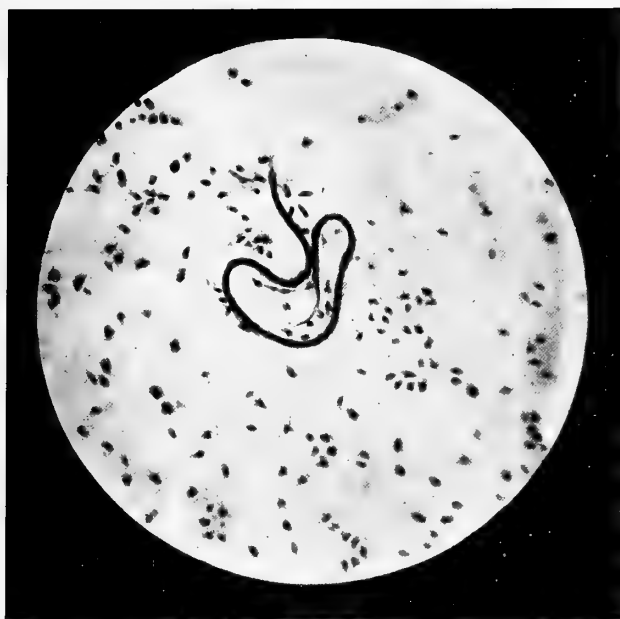


FIG. 1

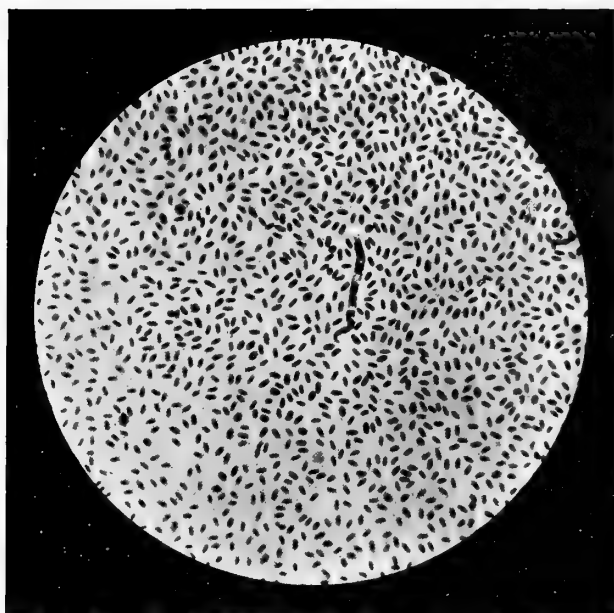


FIG. 2

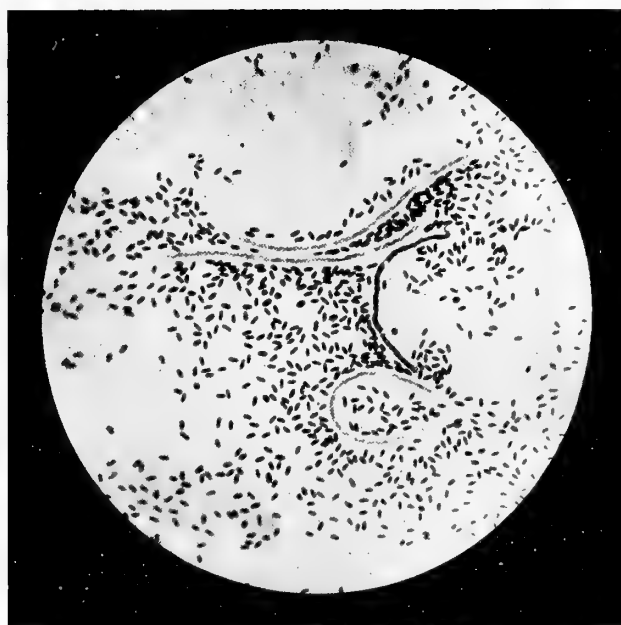


FIG. 3

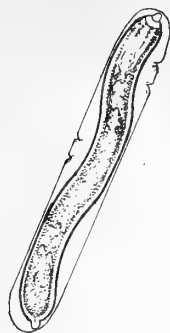


Fig. 1.

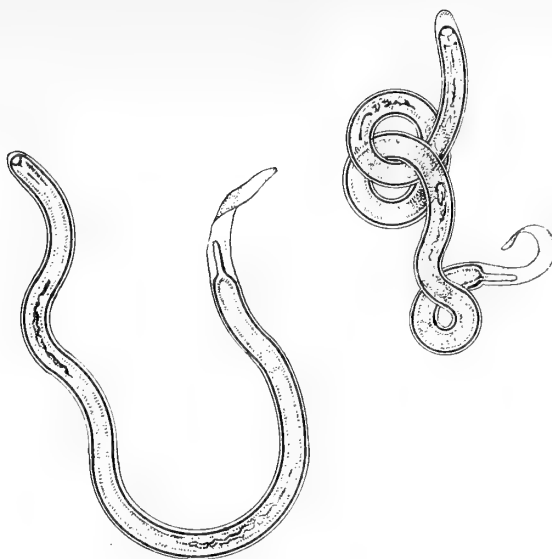


Fig. 2.



Fig. 3.

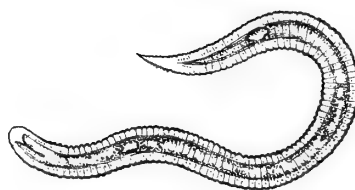


Fig. 4.

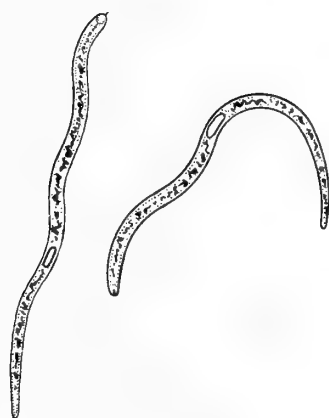


Fig. 5.

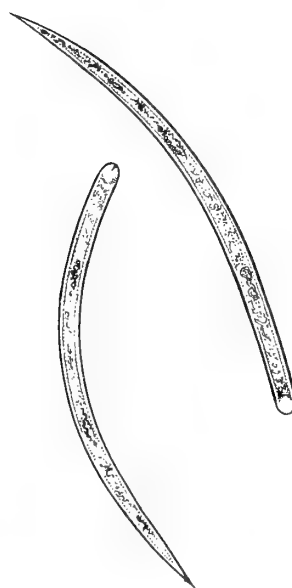


Fig. 6.



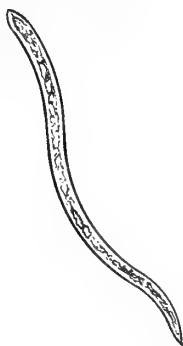


Fig. 7.

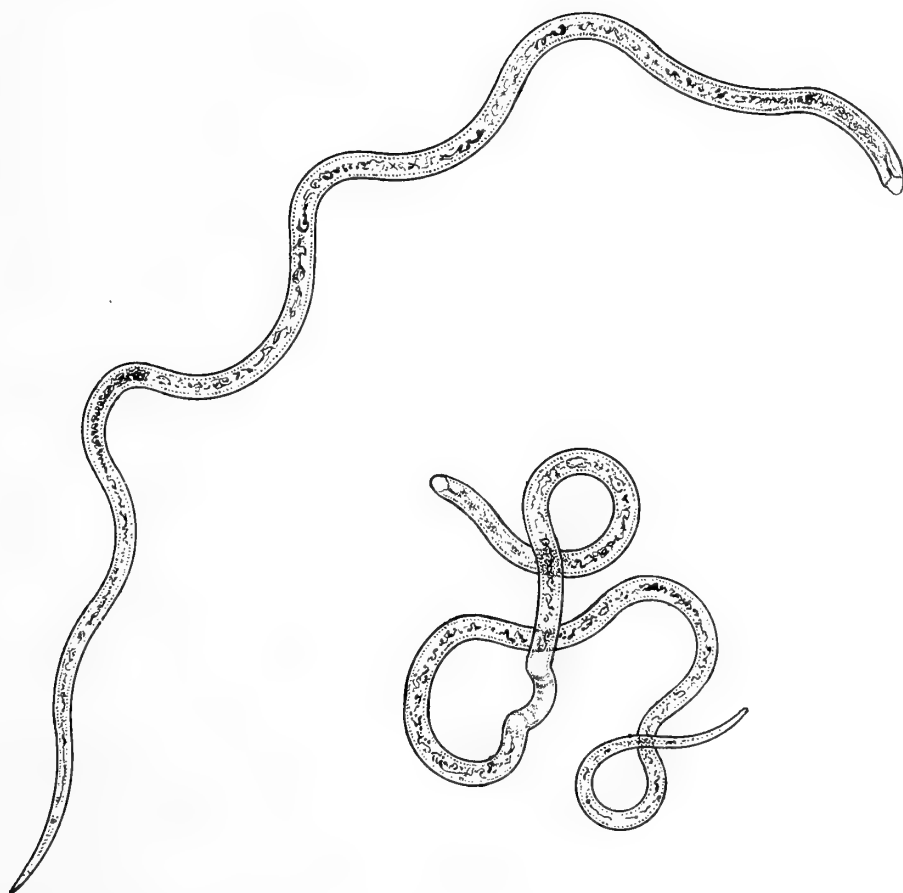


Fig. 8.



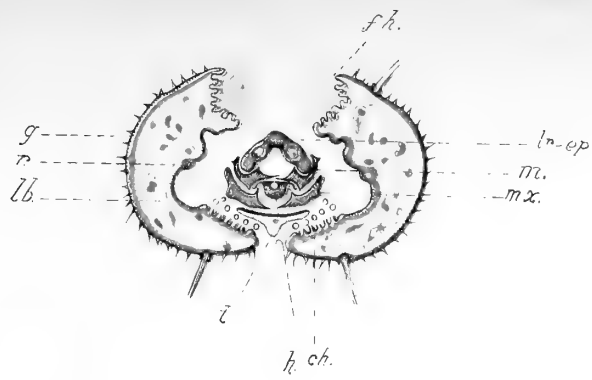


FIG 1.

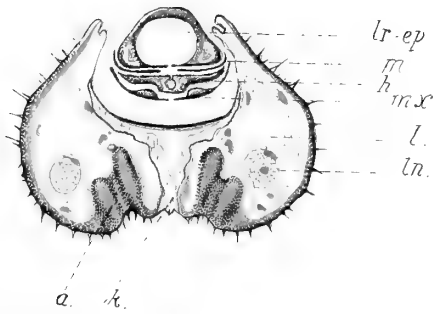


FIG 2

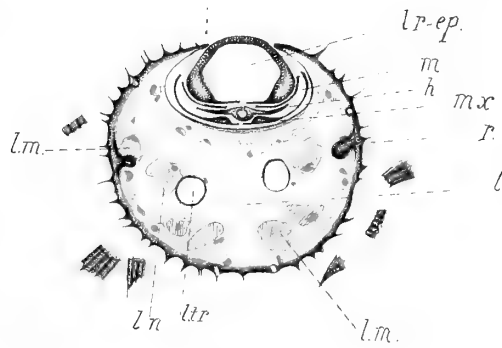
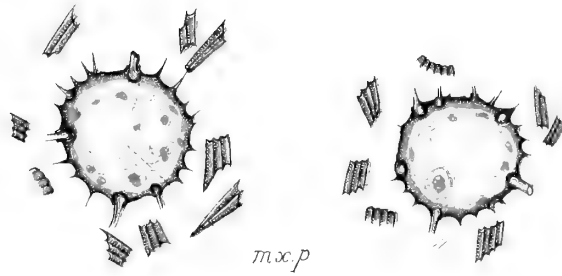


FIG 3



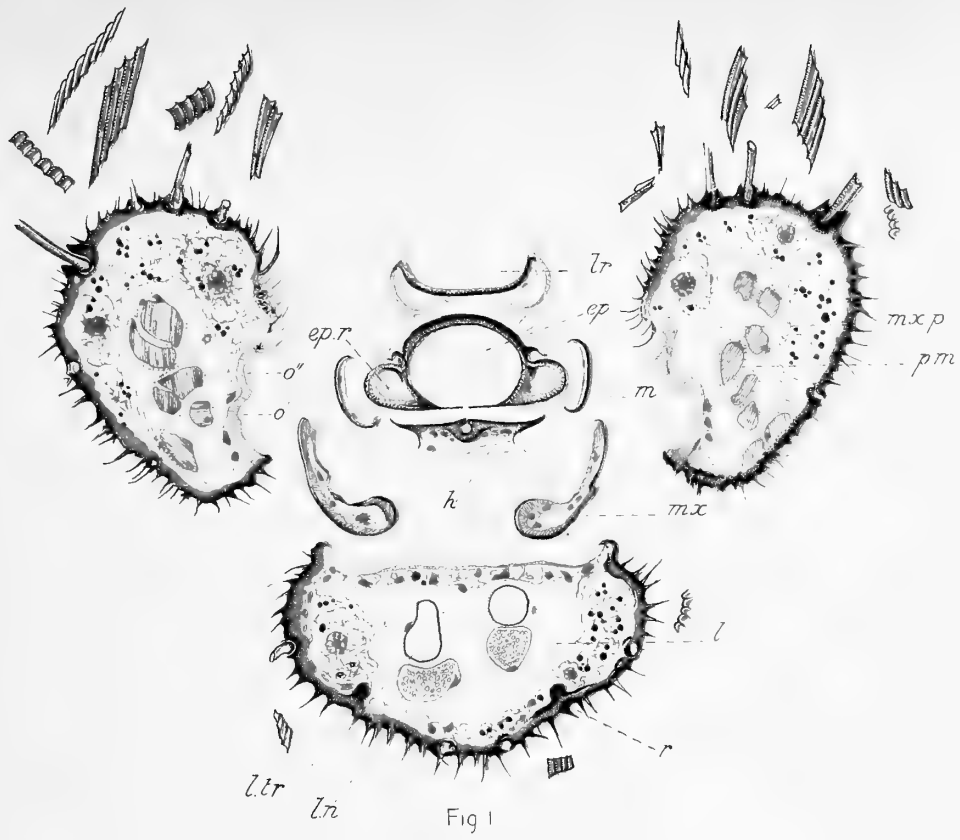


Fig 1

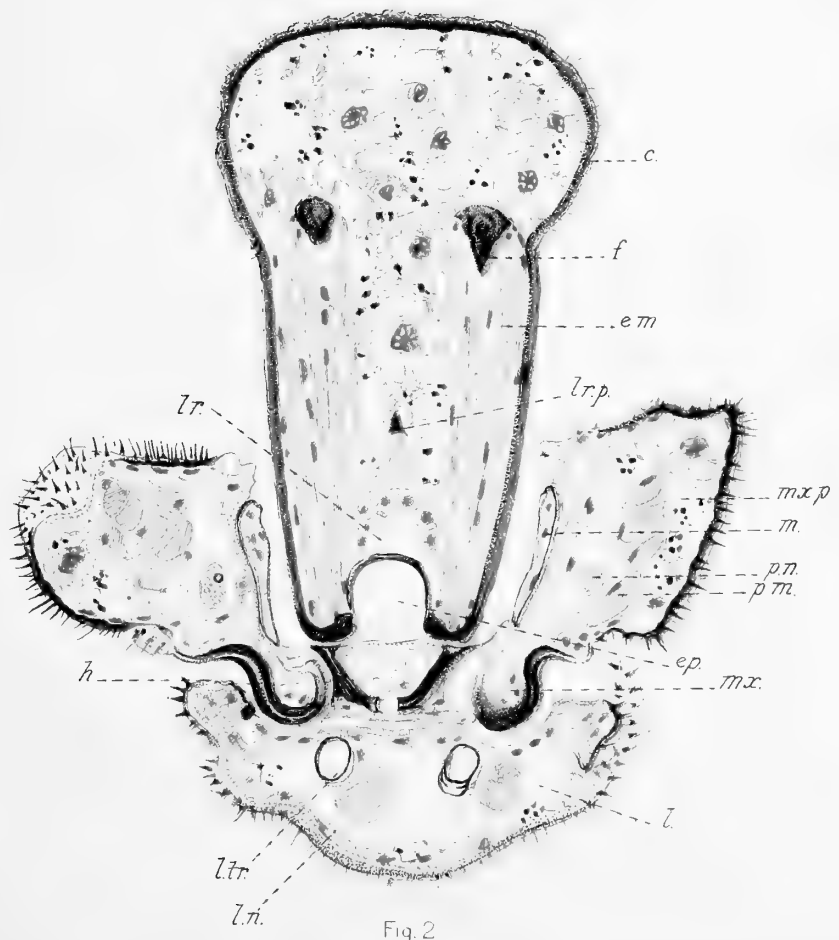
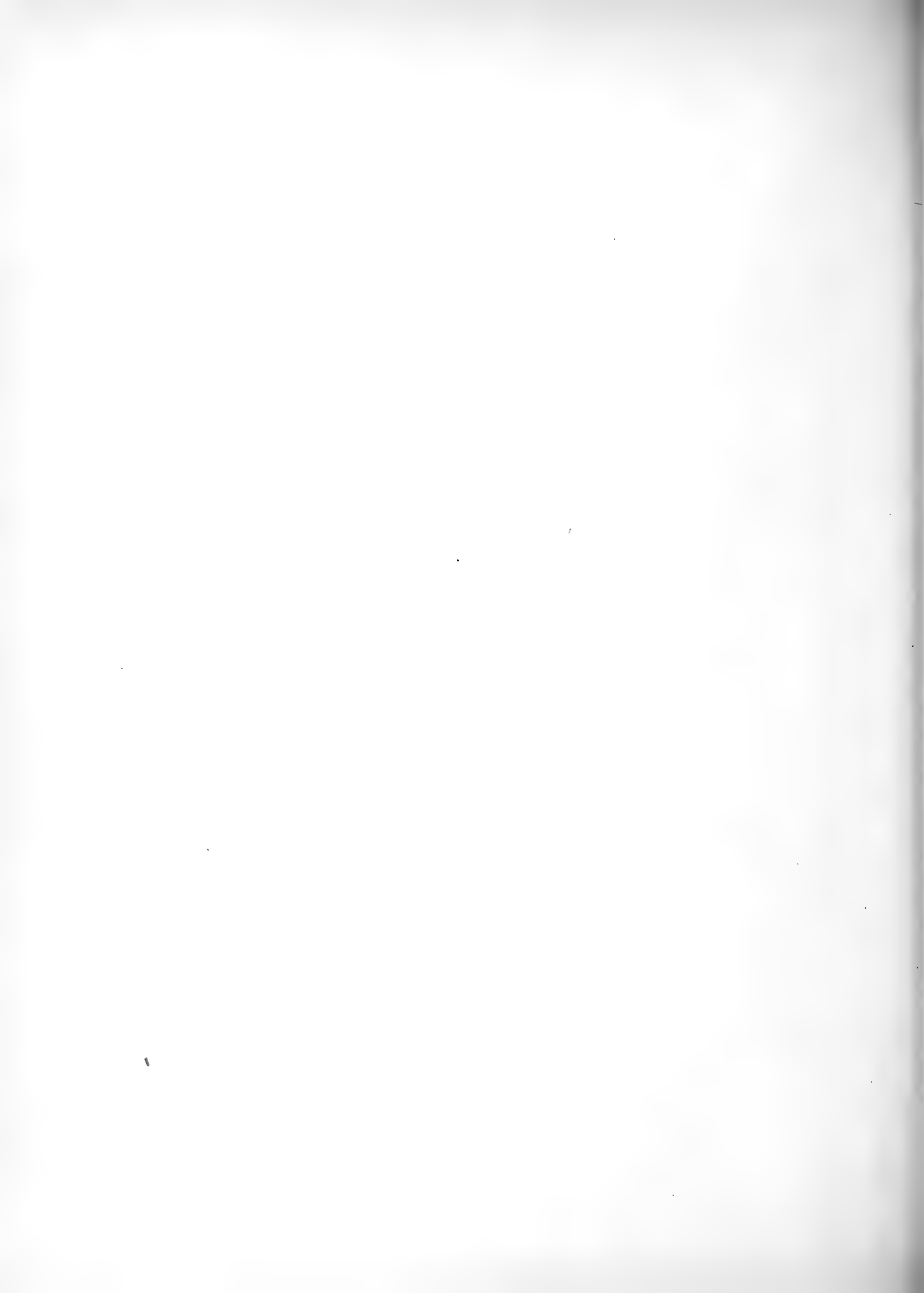
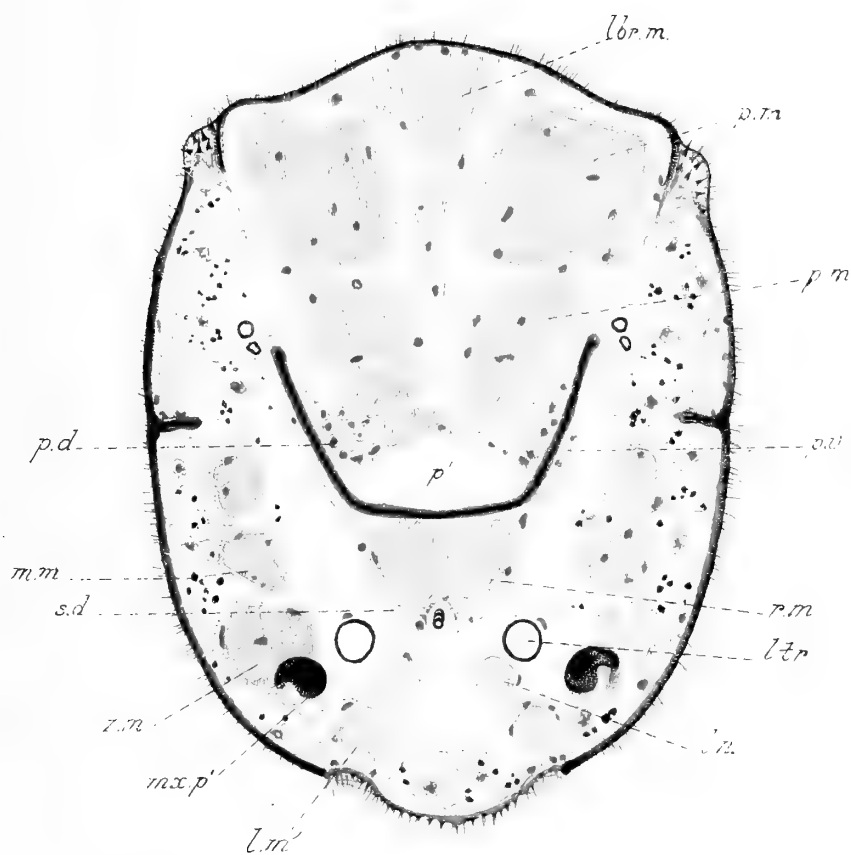


Fig. 2





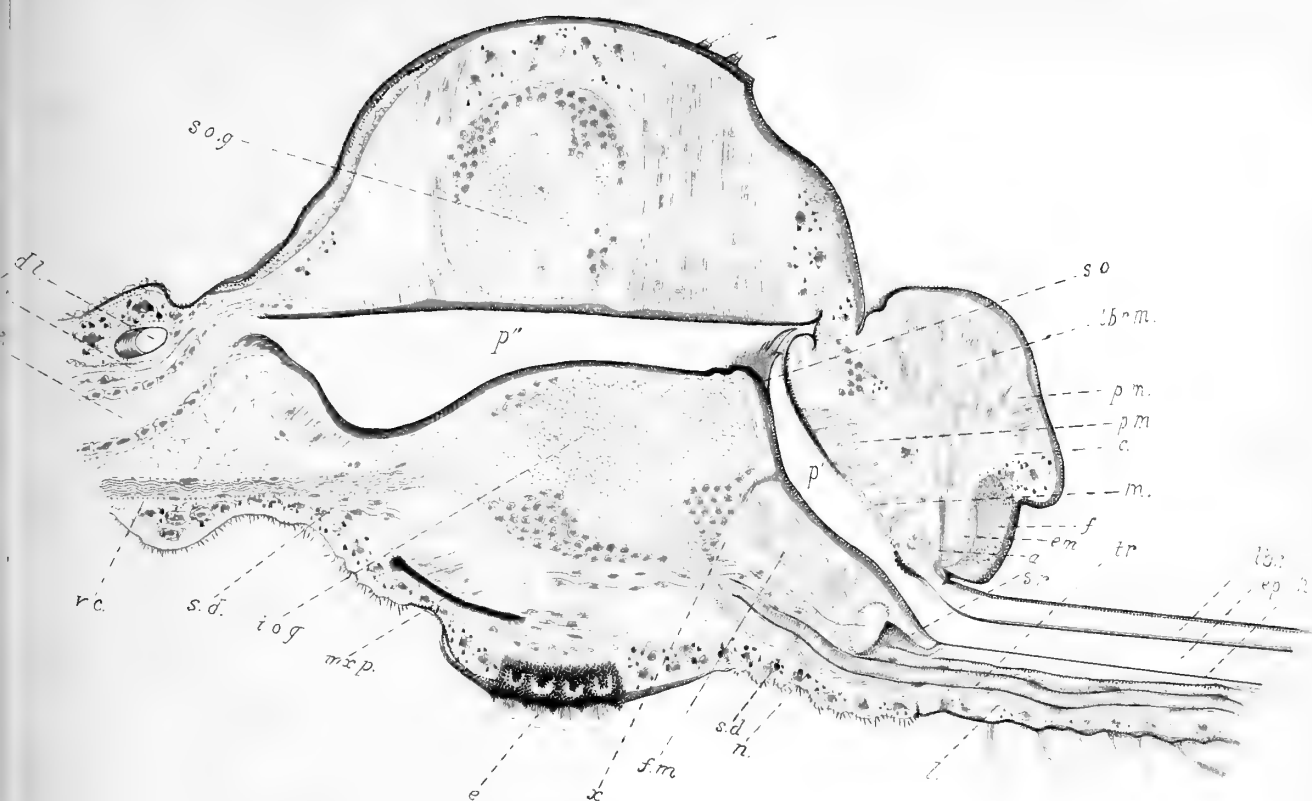


FIG 1

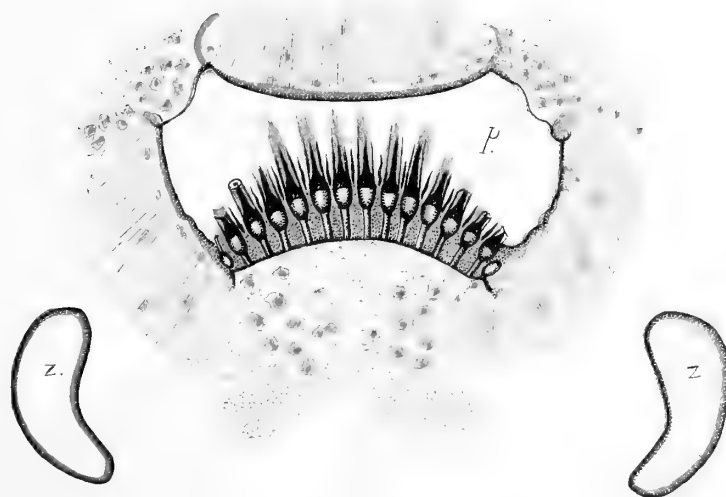


FIG 2.



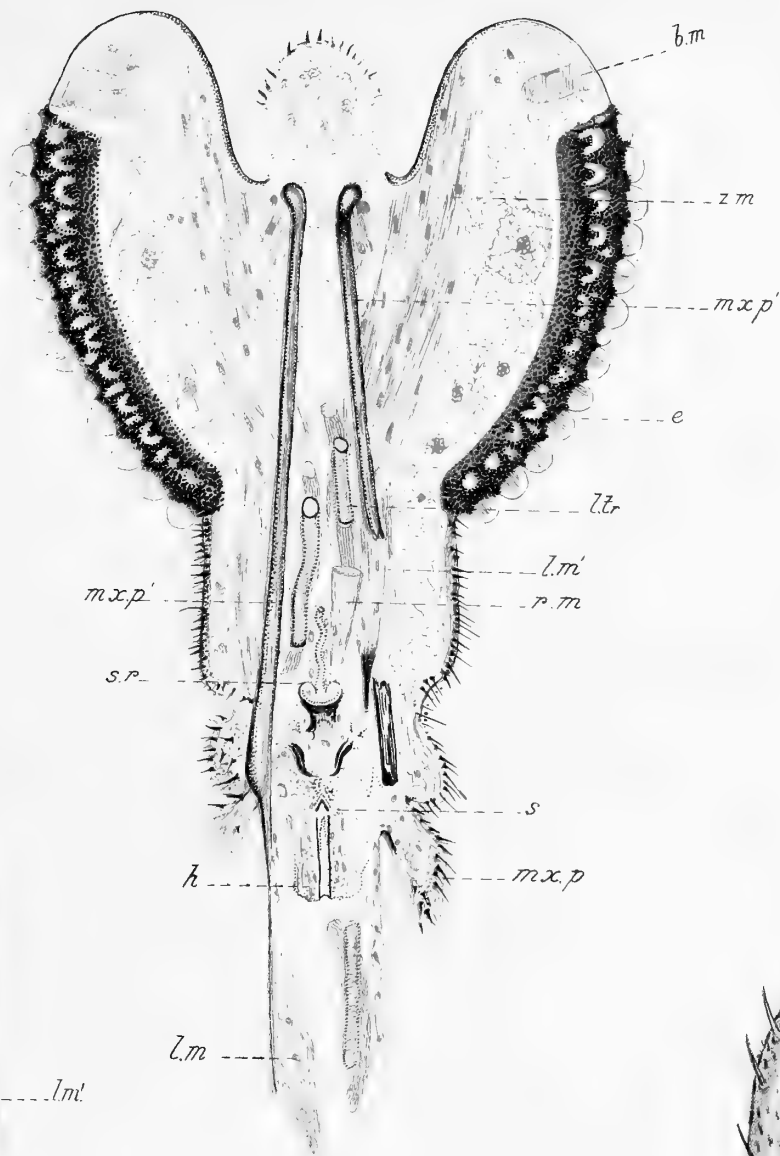


Fig 1

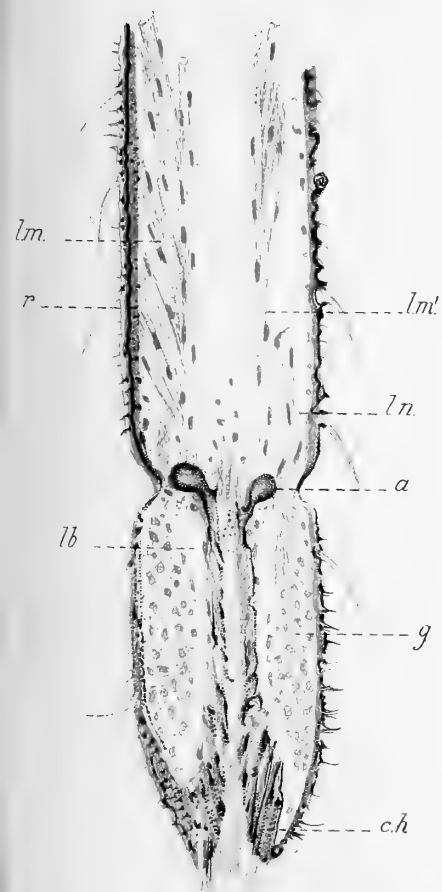


Fig. 2.

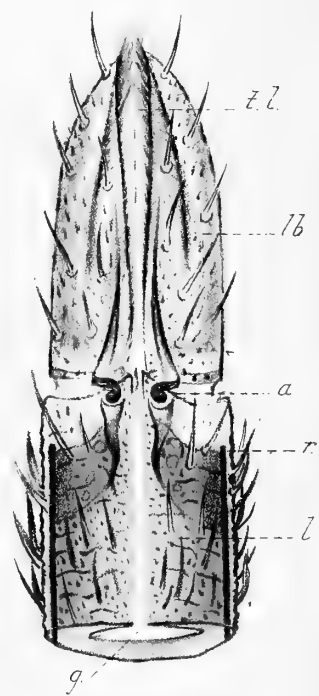


Fig 3

BIBLIOGRAPHY

BIBLIOGRAPHY

COMPLETE LIST OF FILARIAE*

I. LARVAL FORMS

NAME	LITERATURE	HOST	SITE
(a) Mammalia			
<i>F. diurna</i> . Manson	See chapter iv	<i>Homo sapiens</i>	See chapter iv
<i>F. perstans</i> . Manson	" "	<i>Homo sapiens</i>	" "
<i>F. vesperuginis</i> . Linstow	Linstow. Arch. f. Naturg., li, 1885, p. 243	<i>Vesperugo scrotonus</i> (Hameln)	In "long oval" cysts in the intestinal wall
<i>F. irritans</i> . Rivolta	Railliet. Zool. medic. et agric., Paris, 1893, p. 508	<i>Equus caballus</i> . <i>Equus asinus</i>	See chapter ii
(b) Aves			
<i>F. gruis</i> . Linstow	Linstow. Arch. f. Naturg., xli, 1875, p. 197	<i>Ciconia alba</i> ; <i>Grus cinerea</i>	Encysted either in stomach or intestinal wall
<i>F. strigis</i> . Linstow	Linstow. Arch. f. Naturg., xliii, 1877, p. 176; xlv, 1879, p. 173; xlv, 1880, p. 45; xlviii, 1882, p. 1; li, 1885, p. 244	<i>Buteo vulgaris</i> (Hameln) <i>B. lagopus</i> (Hameln) <i>Otus vulgaris</i> (Hameln) <i>Nisus communis</i> (Hameln) <i>Astur palumbarius</i> (Hameln) <i>Bubo maximus</i> (Hameln) <i>Surnia noctua</i> (Hameln) <i>Srix flammea</i> (Hameln) <i>Surnia ulula</i> (Hameln) <i>Lanius excubitor</i> (Hameln)	
(c) Pisces			
<i>F. bicolor</i> . Linstow	Linstow. Arch. f. Naturg., xxxix, 1873, p. 298	<i>Silurius glanis</i> (Hameln)	Under the peritoneal layer of stomach
(d) Arthropoda			
<i>F. stomoxeos</i> . Linstow	Linstow. Arch. f. Naturg., xli, 1875, p. 195	<i>Stomoxys calcitrans</i> (Hameln)	In the proboscis
<i>F. ephemeridarum</i> . Linstow	Linstow. Arch. f. Mikr. Anat., xxxix, 1892, p. 396	<i>Ephemera vulgata</i> (Göttingen) <i>Oligoneura rhenana</i> (Göttingen) <i>Geotrupis sylvaticus</i> (Göttingen) <i>Glomeris limbata</i> (Hameln) <i>Gammarus pulex</i> (Göttingen) <i>Gammarus pulex</i> (Göttingen)	
<i>F. geotrupis</i> . Linstow	Linstow. Arch. f. Mikr. Anat., xlviii, 1896, p. 375		
<i>F. glomeridis</i> . Linstow	Linstow. Arch. f. Naturg., li, 1885, p. 243		
<i>F. pulicis</i> . Linstow	Linstow. Jena. Zeitsch., xxviii, 1893, p. 340		
<i>F. gammari</i> . Linstow	Linstow. Arch. f. Mikr. Anat., xxxix, 1892, p. 325		

II. ORAL APERTURE WITHOUT LIPS

(a) Mammalia

<i>F. bancrofti</i> . Cobbold	See chapter iv	<i>Homo sapiens</i>	See chapter iv
<i>F. loa</i> . Guyot	See chapter iv	<i>Homo sapiens</i>	See chapter iv
<i>F. lentis</i> . Diesing	Diesing. Syst. Helm., ii, 1851, p. 265 Molin. Wien. Sitzber., xxviii, 1858, p. 390 Diesing. Wien. Sitzber., xlii, 1860, p. 702 Cobbold. Entoz. London, 1864, p. 332 De Bonis. Paras. d. corpo umano, Napoli, 1876, p. 129 Davaine. Traité d. Entoz., Paris, 1877, p. 106 Küchenmeister et Zörn. D. Paras d. Mensch, Leipzig, 1881, p. 429	<i>Homo sapiens</i> (Berlin)	See chapter ii
<i>F. labialis</i> . Pane	Railliet. Zool. med. et agric., Paris, 1893, p. 529 Pane. Ann. Accad. d. Aspiranti Natur., Naples, 1864, p. 32 Davaine. Traité d. Entoz., Paris, 1877, p. cvii Küchenmeister u. Zörn. Paras d. Mensch, Leipzig, 1881, p. 430 Railliet. Zool. med. et agric., Paris, 1893, p. 530 Parona. Elminth italiana, Genova, 1894, p. 239	<i>Homo sapiens</i> (Naples)	See chapter ii

* From STOSSICH, *Filarie e Spiroptere*. Trieste, 1897.

NAME	LITERATURE	Host	SITE
<i>F. medinensis</i> . Gmelin	Gmelin. Syst. Natur., 1878, p. 3039 Rudolphi. Entoz. Synops., 1819, p. 3 Lamark. Anim. s. vert., iii, 1840, p. 667 Dujardin. Hist. Nat. d. Helm., 1845, p. 44 Diesing. Syst. Helm., ii, 1851, p. 269 Baird. Catal. of Entoz., London, 1853, p. 5 Molin. Wien. Sitzber., xxviii, 1858, p. 403 Schneider. Monogr. d. Nemat., 1866, p. 85 De Bonis. Paras. d. corpo umano, Naples, 1876, p. 128 Davaine. Traité d. Entoz., Paris, 1877, p. cvii Küchenmeister u. Zürn. D. Paras. d. Mensch, Leipzig, 1881, p. 417 Zürn. Thier. Paras. uns. Haussäuget, Weimar, 1882, p. 249 Blanchard. Anim. Paras., introd. par l'eau, Paris, 1890, p. 71 Railliet. Zool. med. et agric., Paris, 1893, p. 500 Parona. Elminthol italiana, Genova, 1894, p. 239 Valenciennes. Compt. rend Acad. Sc., xliii, 1856, p. 259 Molin. Wien. Sitzber., xxviii, 1838, p. 373 Diesing. Wien. Sitzber., xlii, 1860, p. 686 Diesing. Wien. Sitzber., xliii, 1861, p. 286 Huber. Bibliogr. I. klin. Helminth, München, 1894, p. 245 Diesing. Wien. Sitzber., xlii, 1860, p. 698 Cobbold. Entoz., London, 1864, p. 373 Sonsino. Pr. Verb. d. Soc. Toscana d. sc. nat., 12 mag., 1889	<i>Homo sapiens</i> (Arabia, Persia, Turkestan, India, Guinea, Senegambia, Darfur, Sennaar, Abyssinia, Nubia, Egitto, Curacao, Brazil); <i>Bos taurus</i> , <i>Equus caballus</i> (India); <i>Canis familiaris</i> (Egitto, Buenos Ayres, Curacao, India); <i>Canis lupaster</i> : <i>C. aureus</i> : <i>Felis catus</i> , <i>F. guttata</i> (Kordofan)	See chapter ii
Syn. <i>F. aethiopica</i>			
<i>Dracunculus persarum</i>			
<i>Dracunculus aethiopicus</i>			
<i>Dracunculus medinensis</i>			
<i>F. lymphatica</i> . Treutler	Railliet. Zool. med. et agric., Paris, 1893, p. 530 Rudolphi. Entoz. Synops., 1819, p. 7 and 215 Dujardin. Hist. Nat. d. Helm., 1845, p. 45 Diesing. Syst. Helm., ii, 1851, p. 279 Molin. Wien. Sitzber., xxviii, 1858, p. 418 Condorelli. Roma, 1892 Addario. Ann. d. ottalmolog., xiv, 1885 Condorelli. Filar. apapill. Roma, 1892 Condorelli. " " " " I, 1887, p. 617 Grassi. Centr. f. Bakt. u. Par. I, 1887, p. 617 Calundruccio. Anim. Paras. dell' uomo in Sicilia, 1889, p. 19 Railliet. Zool. medic. et agric. Paris, 1893, p. 528 Parona. Elminthol. italiana. Genova, 1894, p. 239 Quadri. Cpt. rend. d. Congres. Ophthal. d. Bruzel., 1858, p. 153 Condorelli. Filar. apapill. Roma, 1892 Pace. Giorn. d. sc. nat. ed. econ. Palermo, ii, 1866, p. 152 Condorelli. Filar. apapill. Roma, 1892 De Bonis. Paras. d. corpo umano. Napoli, 1876, p. 130 Pabesius. Arch. f. path. Anat. u. Physiol., 1880, p. 158 Condorelli. Filar. apapill. Roma, 1892 Treutler. Observ. Path. Anat., 1793, p. 10 Zeder. Naturg. d. Eingw., 1803, p. 45 Gurlt. Pathol. Anat., i, 1831, p. 347 Gerber. Handb. d. allg. Anat., 1840, p. 211 Diesing. Syst. Helm., ii, 1851, p. 265 Molin. Wien. Sitzber., xxviii, 1858, p. 372 Diesing. Wien. Sitzber., xlii, 1860, p. 701 Moroni. Il. medico. veterinario. Torino, 1864, April Davaine. Traité d. Entoz. Paris, 1877, p. 109 Zürn. Thier Paras. uns. Haussäuget. Weimar, 1882, p. 248 Perroncito. I parass. dell' uomo e. d. anim. Milano, 1882, p. 32 Railliet. Zool. med. et agric. Paris, 1893, p. 527 Parona. Elminthol. italiana. Genova, 1894, p. 241 Grassi e. Calundruccio. Centr. f. Bakt. u. Paras. vii, 1890, p. 18 Railliet. Zool. med. et agric. Paris, 1893, p. 573 Parona. Elminthol. italiana. Genova, 1894, p. 241 Railliet. Zool. med. et agric. Paris, 1893, p. 528 Baird. Catal. of Entoz. London, 1853, p. 5 Dujardin. Hist. Nat. d. Helm., 1845, p. 88 Diesing. Syst. Helm., ii, 1851, p. 225 Molin. Wien. Sitzber., xxviii, 1858, p. 388 Stossich. Boll. Soc. Adriat. di Sc. Nat., xii, 1890, p. 56 Railliet. Zool. med. et agr. Paris, 1893, p. 508 Parona. Elminthol. italiana. Genova, 1894, p. 241	<i>Homo sapiens</i> (Sicily) <i>Equus caballus</i> (Milan) <i>Equus asinus</i>	See chapter ii
Syn. <i>F. hominis bronchialis</i>			
<i>F. apapillocephala</i>			
<i>F. conjunctivae</i>			
<i>F. dubini</i>			
<i>F. inermis</i>			
<i>F. oculi</i>			
<i>F. oculi asini</i>			
<i>F. palpebralis</i>			
<i>F. palpebrarum</i>			
<i>F. peritonaei hominis</i>			
<i>Hamularia lymphatica</i>			
<i>Tentacularia subcompressa</i>			
<i>F. lacrymalis</i> . Gurlt		<i>Bos taurus</i> , <i>Equus caballus</i>	See chapter ii
<i>F. recondita</i> . Grassi		<i>Canis familiaris</i> (Catania)	See chapter ii
<i>F. palpebralis</i> . Wilson		<i>Equus caballus</i>	See chapter ii
<i>F. nasicola</i> . Leuchart		<i>Mustela foina</i> ; <i>M. putorius</i>	In frontal and ethmoid sinuses
Syn. <i>Spiroptera nasicola</i>			
<i>F. acutiuscula</i> . Molin		<i>Dicotyles albirostris</i> (Brazil); <i>D. torquatus</i> (Brazil); <i>Vulpus azarae</i> , <i>Canis familiaris</i> (Venice)	Between the viscera In the pectoral muscles

NAME	LITERATURE	HOST	SITE
<i>F. hyalina</i> . Linstow	Linstow. Arch. f. Naturg., 1890, p. 182	<i>Sorex vulgaris</i> (Göttingen)	Intestine
<i>F. haemorrhagica</i> . Railliet.	Railliet et Moussu. Compt. rend. d. l. Soc. d. Biol., iv, 1892, p. 545	<i>Equus caballus</i>	See chapter ii
	Railliet. Zool. med. et agric. Paris, 1893, p. 505	<i>Equus asinus</i>	
<i>F. immitis</i> . Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 55	<i>Canis familiaris</i> (Europe, U.S.A., Brazil, Australia, Borneo, China, Japan); <i>C. lupus</i> (Japan); <i>C. vulpes</i> , <i>C. brachyurus</i>	In right heart, pulmonary arteries, and sometimes in other veins and arteries; occasionally free in thoracic cavity, in liver and subcutaneous and inter-muscular tissue
	Molin. Wien. Sitzber, xxviii, 1858, p. 384		
	Schneider. Monogr. d. Nematod., 1866, p. 87		
	Railliet. Journ. d. Veter. d. Midi., 1862, p. 49		
	De Silvestri. Il medico veterinario, ser. iii, vol. vi, 1871, p. 343		
	Ercolani. Mem. R. Acad. d. Sc. Bologna, series iii, tom. v, 1874, p. 390		
	Lewis. Quart. Journ. of micr., s. xv, 1875, p. 268		
	Davaine. Traité d. Entoz. Paris, 1877, p. 108		
	Rivolta. Giorn. Anat. fisiol. patol. anim., dom. ix, 1879, p. 17		
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	Railliet. Zool. med. et agric. Paris, 1893, p. 509		
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	Parona. Boll. d. Musei d. R. Univ. d. Genova, 1896, n. 43		
	Galli-Valerio. Moderna Zooiatria, 1897		
Syn. <i>F. canis cordis</i>	Leidy. Proc. Acad. Nat. Sc. Philadelphia, v, 1853, p. 118		
<i>F. papillicauda</i>	Molin. Wien. Sitzber, xxviii, 1858, p. 380		
" "	Diesing. Wien. Sitzber, xlii, 1860, p. 701		
<i>F. flexuosa</i> . Wedl	Wedl. Wien. Sitzber, xix, 1852, p. 122	<i>Cervus elaphus</i> (Vienna)	In subcutaneous tissues
	Molin. Wien. Sitzber, xxviii, 1858, p. 386		
<i>F. crassicauda</i> . Creplin	Linstow. Württemb. naturw. Jahreshette, 1879, p. 328	<i>Balaenoptera rostrata</i> (Rugen)	In the corpus cavernosus of the penis
	Creplin. Nov. Act. Nat. Cur., xiv, 1829, p. 874	<i>Balaena mysticetus</i>	
	Dujardin. Hist. Nat. d. Helm., 1845, p. 50		
	Diesing. Syst. Helm., ii, 1851, p. 264		
	Molin. Wien. Sitzber, xxviii, 1858, p. 374		
	Beneden. Bull. Acad. Roy. Bruxelles, ser. ii, tom. xxix, 1870, p. 356		
<i>F. quadrispina</i> , Diesing	Diesing. Syst. Helm., ii, 1851, p. 271	<i>Mustela martes</i> (Pavia)	Under the skin, in the pericardial sac; in the cavity of the abdomen
	Schneider. Monogr. d. Nemat., 1866, p. 85	<i>M. foinea</i> (Trieste, Cittanova in Istria, Padua, Genova), <i>M. putorius</i> (Padua), <i>Hystrix cristata</i> (Senaar), <i>Gulo barbatus</i> (Brazil), <i>Galictis barbara</i>	
	Stossich. Boll. Soc. Adriat. di Sc. Nat. Trieste, vii, 1890, p. 56; xiv, 1893, p. 85; xvii, 1896, p. 122		
Syn. <i>F. perforans</i>	Molin. Wien. Sitzber, xxviii, 1858, p. 387		
	Molin. " " xxx, 1858, p. 155		
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<i>F. martis</i>	Gmelin. Syst. Nat. Lipsiae, 1788, p. 3040		
	Zeder. Naturg. d. Eingw., 1803, p. 38		
<i>F. mustelorum subcutanea</i>	Rudolphi. Entoz. Synops., 1819, p. 7 and 216		
<i>F. australis</i> . Linstow	Linstow. Arch. f. Mikr. Anat., xlix, 1897, p. 610	<i>Petrogale penicillata</i> (Australia)	Visceral cavity
(b) Aves			
<i>F. calamiformis</i> . Schneider	Schneider. Monogr. d. Nemat., 1866, p. 90	<i>Psittacus aestivus</i> (Brazil)	Above the tendons of the feet
<i>F. mazzeantii</i> . Railliet.	Railliet. Zool. med. et agric. Paris, 1893, p. 532	<i>Columba domestica</i>	Under the skin of the neck
<i>F. schneideri</i> . Stossich	Schneider. Monogr. d. Nemat., 1866, p. 101	<i>Falco subbuteo</i> (Berlin)	Stomach
<i>F. obtusoraudata</i>	Linstow. Württemb. Naturw. Jahresh, 1879, p. 325	<i>Tetrao urogallus</i>	Under the skin
<i>F. urogalli</i> . Linstow	Magalhães. Revista Brazil d. Medicina, i, 1888, p. 5	<i>Gallus domesticus</i> (China, Rio Janeiro)	Orbital cavity
<i>F. mansonii</i> . Cobbold	Railliet. Zool. med. et agric., Paris, 1893, p. 533		
	Magalhães. Bull. Soc. Zool. de France, xx, 1895, p. 241		

NAME	LITERATURE	HOST	SITE
<i>F. foveolata</i> . Molin	Molin. Wien. Sitzber, xxviii, 1852, p. 375 Linstow. Arch. f. Naturg., xlv., 1879, p. 172 Stossich. Hist. Natur., Croat, vi, 1891, p. 217 Stossich. Hist. Natur., Croat, vii, 1892, p. 72	<i>Circus cyaneus</i> ; <i>Falco peregrinus</i> (Trieste, Venice, Hameln); <i>F. lunarius</i> ; <i>F. lithofalco</i> ; <i>Nisus communis</i> (Venice); <i>Corvus frugilegus</i> (Switzerland); <i>Thamnophilus stagurus</i> (Brazil)	In pleural and abdominal cavities
<i>F. nodulosa</i> . Rudolphi	Diesing. Syst. Helm., ii, 1851, p. 274 Molin. Wien. Sitzber, xxviii, 1858, p. 409 Schneider. Monogr. d. Nemat., 1866, p. 91 Linstow. Arch. f. Naturg., xlix, 1883, p. 287 " Verm. Mosca, 1886, p. 12 Leidy. Proc. Acad. Nat. d. Sc. Philadelphia, 1886, p. 309 Parona. Elmintol. sarda Genova, 1887, p. 87 " Ann. Museo civico di Genova, 1887, p. 495 Stossich. Boll. Soc. Adriat. disc. Nat. Trieste, xii, 1890 " Soc. Hist. Nat. Croat., vii, 1892, p. 72 " Boll. Soc. Adriat. di Sc. Nat. Trieste, xiv, 1893 Parona. Elmintol. italiana. Genova, 1894, p. 242 Stossich. Boll. Soc. Adriat. di sc. Nat. Trieste, xvii, 1896, p. 122	<i>Lanius collurio</i> (Trieste, Venice, Berlin, Turkestan); <i>L. rufus</i> (Cagliari, Genova); <i>L. auriculatus</i> (Cagliari, Genova); <i>L. minor</i> ; <i>Collurio ludovicianus</i> (Florida)	Under the skin of the cranium and back, and in oesophageal wall
Syn. <i>Tentacularia cylindrica</i> <i>F. collurionis pulmonalis subcutanea</i>	Zeder. Naturg. d. Eingw., 1803, p. 45 Rudolphi. Entoz. Synops., 1819, pp. 8 and 217 " " " 1819, pp. 8 and 217		
<i>F. obtusocaudata</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 634 Dujardin. Hist. Nat. d. Helm., 1845, p. 55 Diesing. Syst. Helm., ii, 1851, p. 277 Molin. Wien. Sitzber, xxviii, 1858, p. 413 Linstow. Württemb. naturw. Jahresh., 1879, p. 327 " Arch. f. Naturg., xlix, 1883, p. 284 " Verm. Mosca, 1886, p. 10 Parona. Ann. Museo civico di Genova, xxvii, 1889, p. 762 Diesing. Wien. Sitzber, xlii, 1860, p. 710	<i>Lanius rufus</i> ; <i>L. minor</i> ; <i>Pernix leucostriata</i> ; <i>Picus flavescens</i> ; <i>P. robustus</i> ; <i>P. lineatus</i> ; <i>P. passerinus</i> ; <i>P. aurulentus</i> ; <i>P. leucolaemus</i> ; <i>P. iumana</i>	In the muscles of the neck, and in the thoracic cavity
Syn. <i>Monopetalonema obtuse-caudatum</i> <i>F. spermospizae</i> . Linstow. <i>F. bhamoensis</i> . Parona <i>F. paronai</i> . Stossich Syn. <i>F. sp.</i> <i>F. sp.</i>	Linstow. Arch. f. Naturg., xlv, 1879, p. 171 Parona. Ann. Museo civico di Genova, 1890, p. 777 Parona. Ann. Museo civico di Genova, 1885, p. 433 Linstow. Arch. f. Naturg., 1891, p. 300 Örley. Ann. Mag. of Nat. Hist., 1882, p. 312 Linstow. Arch. f. Naturg., 1891, p. 300 Wedl. Wien. Sitzber, xix, 1856, p. 126 Molin. Wien. Sitzber, xxviii, 1858, p. 374 Diesing. Wien. Sitzber, xlii, 1860, p. 701 Ralliet. Zool. med. et agric. Paris, 1893, p. 532 Linstow. Arch. f. Naturg., xlix, 1883, p. 285 Linstow. Verm. Mosca, 1886, p. 10 Linstow. Arch. f. Naturg., 1891, p. 293 Stossich. Boll. Soc. Adriat. di sc. nat. Trieste, xvii, 1896, p. 122	<i>Spermospiza guttata</i> <i>Acridotheres albocinctus</i> (Birmanja)	Internal cavities In abdominal cavity
<i>F. ecaudata</i> . Örley	Örley. Ann. Mag. of Nat. Hist., 1882, p. 312 Linstow. Arch. f. Naturg., 1891, p. 300	<i>Buceros nasutus</i> (Sou'an)	Kidney
<i>F. clava</i> . Wedl	Wedl. Wien. Sitzber, xix, 1856, p. 126 Molin. Wien. Sitzber, xxviii, 1858, p. 374 Diesing. Wien. Sitzber, xlii, 1860, p. 701 Ralliet. Zool. med. et agric. Paris, 1893, p. 532	<i>Lamprolornis aeneus</i>	See chapter ii
<i>F. tricuspis</i> . Fedtschenko	Linstow. Arch. f. Naturg., xlix, 1883, p. 285 Linstow. Verm. Mosca, 1886, p. 10 Linstow. Arch. f. Naturg., 1891, p. 293 Stossich. Boll. Soc. Adriat. di sc. nat. Trieste, xvii, 1896, p. 122	<i>Corvus cornix</i> (Venice, Padua, Vienna, Turkestan); <i>C. corone</i> (Vienna), <i>C. frugilegus</i> (Padua, Trieste, Vienna); <i>C. corax</i> (Vienna); <i>C. monedula</i> (Vienna); <i>Pyrrhocorax alpinus</i> (Vienna); <i>Pica caudata</i> (Vienna); <i>Garrulus glandarius</i> (Vienna); <i>Nucifraga caryocatactes</i> (Vienna); <i>Sturnella ludoviciana</i> ; <i>Sylvia atricapilla</i> (Zagabria); <i>Poecile palustris</i> (Zagabria); <i>Corvus cyanomelas</i> ; <i>Hirundo rustica</i> ; <i>Alauda arvensis</i> (Trieste); <i>Lullula arborea</i> (Trieste); <i>Acridotheres tristis</i> (East Indies); <i>A. ginginianus</i> (East Indies)	Abdominal cavity
Syn. <i>F. unguiculata</i>	Rudolphi. Entoz. Synops., 1819, pp. 4 and 209 Dujardin. Hist. Nat. d. Helm., 1845, p. 54 Diesing. Syst. Helm., ii, 1851, p. 267 Molin. Wien. Sitzber, xxviii, 1858, p. 378 Stossich. Soc. Hist. Nat. Croat., v, 1890, p. 130 Stossich. Soc. Hist. Nat. Croat., vi, 1891, p. 217 Zeder. Natur. d. Eingw., 1803, p. 39	<i>Paradisea apodu</i> (Aru Island); <i>Cyanops ramsayi</i> (Tenasserim)	Subcutaneous, and in internal cavities
<i>F. monticelliana</i> <i>F. ninnii</i> <i>F. alaudae</i>		<i>Turdus cyaneus</i> (Argo)	
<i>F. flabellata</i> . Linstow	Linstow. Zool. of the voyage of H.M.S. Challenger, vol. xxiii, part lxxi, London, 1888, p. 9 Linstow. Arch. f. Naturg., 1891, 300 Parona. Ann. Museo civico di Genova, 1890, p. 777 Schneider. Monogr. d. Nemat., 1866, p. 92 Linstow. Arch. f. Naturg., 1891, p. 300 Zeder. Naturg. d. Eingw., 1803, p. 36 Rudolphi. Entoz. Synops., 1819, p. 4 Dujardin. Hist. nat. d. Helm., 1845, p. 53 Diesing. Syst. Helm., ii, 1851, p. 267 Baird. Catal. of Entoz. London, 1853, p. 6 Molin. Wien. Sitzber, xxviii, 1858, p. 397 Diesing. " " xlii, 1860, p. 702	<i>Hirundo rustica</i> (Vienna, Greifswald, Rennes); <i>H. urbica</i> (Genova, Vienna, Turkestan); <i>H. riparia</i> (Vienna); <i>H. versicolor</i> (Brazil); <i>Progne purpurea</i> ; <i>Myothera caudacuta</i>	Abdominal cavity
<i>F. pungens</i> . Schneider			
<i>F. obtusa</i> . Rudolphi			

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V

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<i>F. turdi atrogalaris</i> . Linstow	Linstow. Arch. f. Naturg., xlix, 1883, p. 288 Linstow. Verm. Mosca, 1886, p. 13	<i>Turdus atrogularis</i> (Turkestan)	
(c) Reptilia			
<i>F. macrophallos</i> . Parona	Parona. Ann. Museo civico d. Genova, 1890, p. 778	<i>Hydrosaurus salvator</i> (Birmanja)	Between the abdominal muscles
<i>F. emmae</i> . Stossich Syn. <i>F. sp.</i>	Parona. Ann. Museo civico di Genova, 1890, p. 778	<i>Catotes emma</i> (Burmah)	
<i>F. dahomensis</i> . Neumann	Neumann. Bull. Soc. Zool. de France, xx, 1895, p. 123	<i>Python natalensis</i> (Dahomey)	In the subcutaneous tissue of the abdominal wall
(d) Pisces			
<i>F. denticulata</i> . Rudolphi Syn. <i>Cochlus inermis</i> <i>Liorhynchus denticulatus</i>	Schneider. Monog. d. Nemat., 1866, p. 102 Zeder. Naturg. d. Eingw., 1803, p. 50 Rudolphi. Entoz. Synops, 1879, pp. 62 and 307 Bremser. Icon. Helminth, 1824 Lamark. Anim. s. vert., iii, 1840, p. 646 Dujardin. Hist. Nat. d. Helm., 1845, p. 284 Diesing. Syst. Helm., ii, 1851, p. 247	<i>Anguilla vulgaris</i>	Stomach
<i>F. echinata</i> . Linstow <i>F. obturans</i> . Prenant	Linstow. Arch. f. Naturg., xlv, 1878, p. 235 Prenant. Bull. Soc. sc. Nancy (2), vii, 1888, p. 215	<i>Alburnis lucidus</i> (Hameln) <i>Esox lucius</i> (Nancy)	Intestine Bronchial artery

III. BUCCAL CAVITY HAS NO LIPS, BUT IS SURROUNDED BY A CHITINOUS RING

(a) Mammalia

<i>F. equina</i> . Abildgaard	Blanchard. Ann. d. Sc. Nat., ser. iii, tom. xi, p. 154 Railliet. Zool. med. et agric. Paris, 1893, p. 524 Neumann. Revue vétérin., xxii, 1897, p. 75 Gmelin. Syst. Natur., 1789, p. 3039 Zeder. Naturg. d. Eingw., 1803, p. 37 Anderson. Edinb. Medic. and Surg. Journal, ii, 1805, p. 306 Sick. Med. Jahrb. d. k. k. österr. Staat, ii, 1813, p. 174 Greve. Krankh. d. Hausth., 1818, p. 174 Rudolphi. Entoz. Synops., 1819, pp. 6 and 213 Bremser. Vers. intest. d. l'hom., 1824, p. 123 Bremser. Icon. Helminth, 1824 Gurlt. Path. Anat. i, 1831, p. 348 Nordmann. Microgr. Beiträge, 1832, p. 11 Siebold. Arch. f. Naturg., 1837, p. 255 Lamark. Anim. s. vert., iii, 1840, p. 668 Dujardin. Hist. Nat. d. Helm., 1845, p. 49 Diesing. Syst. Helm., ii, 1851, p. 272 Baird. Catal. of Entoz. London, 1853, p. 5 Wedl. Wien. Sitzber., xvii, 1855, p. 307; xix, 1856, p. 56 Leidy. Proc. Acad. Nat. Sc., Philadel., viii, 1856, p. 55 Molin. Wien. Sitzber., xxviii, 1858, p. 405 Schneider. Monogr. d. Nematod., 1866, p. 86 Panizza. Il medico veterinario. Torino, 1869, p. 193 Davaine. Traité d. Entoz. Paris, 1877, p. cix Zürn. Their. Paras. uns. Haussäuget. Weimar, 1882, p. 242 Lange. Deutsch. Zeitschr. f. Thier medicin, viii, 1882, p. 71 Linstow. Arch. f. Naturg., xlix, 1883, p. 284 Linstow. Verm. Mosca, 1886, p. 9 De Silvestri. Giorn. d. medic. veterin. prat. Torino, xxxvi, 1887, p. 429 Blanchard. Bull. Soc. Zool. France, xii, 1887 Parona. Ann. Museo civico di Genova, 1887, p. 494 Deupser. Zool. Anzeiger, 1892, n. 388 Parona. Elmintol. italiana. Genova, 1894, p. 241 Rudolphi. Entoz. Synops., 1819, p. 8 Molin. Wien. Sitzber., xxviii, 1858, p. 421 Schneider. Monogr. d. Nematod., 1866, p. 103 Linstow. Arch. f. Naturg., 1885, p. 241 Linstow. Zool. Jahrb., iii, 1887, p. 112 Rudolphi. Entoz. Synops, 1819, pp. 24 and 241 Dujardin. Hist. Nat. d. Helm., 1845, p. 86 Diesing. Syst. Helm., ii, 1851, p. 213 Baird. Catal. of Entoz. London, 1853, p. 2	<i>Equus caballus</i> (Italy); <i>E. asinus</i> (Milan); <i>E. mulus</i> ; <i>Bos taurus</i> ; <i>B. bubalus</i>	In peritoneal cavity, testicle, pleural cavity, lungs, between the meninges, in liver, aqueous humour
<i>F. bubali</i>			
<i>F. strumosa</i> . Rudolphi		<i>Talpa europaea</i> (Padua, Sondrio, Rennes, Vienna, Halle, Hameln, Greifswald, Ireland)	Stomach and intestines, and in pedunculated tumours on external coat of stomach
Syn. <i>Spiroptera strumosa</i>		(Larval form encysted in fat bodies of <i>Cetonia aurata</i>)	

NAME	LITERATURE	HOST	SITE
<i>F. strumosa</i> . Rudolphi—contd.	Molin. Wien. Sitzber, xxx, 1858, p. 152; xxxviii, 1859, p. 933 Diesing. Wien. Sitzber, xlii, 1860, p. 677 Molin. Denkschr. Wien. Akad., xix, 1861, p. 300 Parona. Elmintol. italiana. Genova, 1894, p. 246 Galli-Valerio. Moderno Zootatro, 1897 Gmelin. Syst. Nat., p. 3032 Zeder. Naturg. d. Eingw., 8803, p. 106 Blanchard. Ann. d. Sc. Nat., ser. iii, tom. xi, p. 162 Alessandrini. Nuovi annali. di sc. nat. Bologna, i, 1838, p. 1 Perroncito. I paras. dell'uomo e d. anim. Milano, 1882, p. 326 Railliet. Zool. med et agric. Paris, 1893, p. 526 Parona. Elmintol italiana. Genova, 1894, p. 241 Galli-Valerio. Moderno Zootatro, 1897 Rudolphi. Entoz. Synops, 1819, p. 8 Dujardin. Hist. Nat. d. Helm., 1845, p. 49 Diesing. Syst. Helm., ii, 1851, p. 274 Molin. Wien. Sitzber, xxviii, 1858, p. 405 Diesing. Wien. Sitzber, xlii, 1860, p. 703 Schneider. Monogr. d. Nemat., 1866, p. 86	<i>Bos taurus</i> ; <i>Cervus elaphus</i> ; <i>C. columbianus</i> ; <i>C. virginianus</i> ; <i>C. capreolus</i> ; <i>C. rufus</i> ; <i>C. nambi</i> ; <i>C. simplicicornis</i>	Peritoneal cavity
<i>Ascaris talpae</i> <i>Fusaria convoluta</i> <i>Spirura talpae</i>			
<i>F. labiato-papillosa</i> . Alessandrini			
Syn. <i>F. cervi</i> <i>F. cervina</i> <i>F. terebra</i>			
(b) Aves			
<i>F. dehiscens</i> . Schneider	Monogr. d. Nemat., 1866, p. 91	<i>Strix striata</i> (Dongola)	
<i>F. brasiliiana</i> . Stossich			
Syn. <i>P. insignis</i>	Schneider. Monogr. d. Nematod., 1866, p. 91	<i>Picus sp.</i> (Brazil)	Under the skin of the neck
<i>F. foveata</i> . Schneider	Schneider. Monogr. d. Nemat., 1866, p. 90	<i>Otus brachyotus</i> (Brazil)	
<i>F. labiata</i> . Creplin	Blanchard. Ann. d. Sc. Nat., ser. iii, tom. xi, p. 157 Nathusius. Arch. f. Naturg., iii, 1837, p. 53 Siebold. Arch. f. Naturg., 1838, p. 292 Dujardin. Hist. nat. d. Helm., 1845, p. 57 Diesing. Syst. Helm., ii, 1851, p. 276 Molin. Wien. Sitzber, xxviii, 1858, p. 414 Schneider. Monogr. d. Nematod., 1866, p. 89 Stossich. Boll. Soc. Adriat. di sc. Nat. Trieste, xii, 1890, p. 56 Parona. Elmintol italiana. Genova, 1894, p. 243 Condorelli. Boll. Soc. romana per gli studi zoolog., iv., 1895, pp. 93 and 208 Rudolphi. Entoz. Synops., 1819, pp. 9 and 218 Dujardin. Hist. Nat. d. Helm., 1845, p. 57 Diesing. Syst. Helm., ii, 1851, p. 282 Molin. Wien. Sitzber, xxviii, 1858, p. 428 Parona. Elmintol italiana. Genova, 1894, p. 243 Gmelin. Syst. Nat., p. 3040 Zeder. Naturg. d. Eingw., 1803, p. 39 Rudolphi. Entoz. Synops., 1819, p. 9 Dujardin. Hist. Nat. d. Helm., 1845, p. 57 Diesing. Syst. Helm., ii, 1851, p. 282 Molin. Wien. Sitzber, xxviii, 1858, p. 428 Parona. Elmintol italiana. Genova, 1894, p. 243 Diesing. Wien. Sitzber, xlii, 1860, p. 708	<i>Ciconia nigra</i> (Greifswald, Posen, Paris, Venice, Pavia, Umbria); <i>C. alba</i>	Thoracic and abdominal cavities
Syn. <i>F. ardeae nigrae</i>			
<i>F. ciconiae</i>			
<i>Dicheilonema labiatum</i>			

IV. BUCCAL ORIFICE WITH TWO LIPS

(a) Mammalia

<i>F. gastrophila</i> . Mueller	Mueller. Arch. d. Naturg., lx, 1894, p. 113	<i>Felis domestica</i> (Bavaria)	In neighbourhood of end of oesophagus and cardia of stomach
<i>F. radula</i> . Schneider	Schneider. Monogr. d. Nematod., 1866, p. 98	<i>Paradoxurus philippinensis</i>	Stomach

(b) Aves.

<i>F. horrida</i> . Diesing	Diesing. Syst. Helm., ii, 1851, p. 278 Diesing. Denkschr. Wien. Akad., 1857, p. 19 Molin. Wien. Sitzber., xxviii, 1858, p. 416 Schneider. Monogr. d. Nemat., 1866, p. 89 Linstow. Arch. f. Naturg., xlv, 1880, p. 46 Leidy. Proc. Acad. Nat. Sc. Philadelph., 1884, p. 47 Linstow. Arch. f. Mikr. Anat., xlix, 1897, p. 613 Diesing. Wien. Sitzber., xlii, 1860, p. 709	<i>Rhea americana</i> (Brazil)	Stomach; thoracic and abdominal cavities, muscles, under the skin, and in ovum
Syn. <i>Dicheilonema horridum</i>			

NAME	LITERATURE	HOST	SITE
<i>F. paradiseae</i> . Linstow	Linstow. The Zool. of the voyage of H.M.S. Challenger, Entoz., 1888, p. 11	<i>Paradisea apoda</i> (Aru Island)	
<i>F. tridentata</i> . Linstow	Linstow. Arch. f. Naturg., xliii, 1877, pp. 10 and 175	<i>Colymbus articus</i> (Hamelu) <i>Larus ridibundus</i>	Intestine Oesophagus
<i>F. rotundata</i> . Linstow	Linstow. Arch. f. Naturg., xlix, 1883, p. 283 Linstow. Verm. Mosca., 1866, p. 8	<i>Otis macquini</i> (Turkestan)	
<i>F. recta</i> . Linstow	Linstow. Württemb. Naturw. Jahresh., 1879, p. 324	<i>Podiceps cristatus</i>	In stomach wall
<i>F. coelebs</i> . Linstow	Linstow. Württemb. Naturw. Jahresh., 1879, p. 326	<i>Lanius rufus</i>	In stomach wall
<i>F. capitella</i> . Schneider	Schneider. Monogr. d. Nematod., 1866, p. 96	<i>Coracius garrula</i>	In stomach wall

(c) Pisces

<i>F. conoura</i> . Linstow	Linstow. Arch. f. Naturg., li, 1885, p. 242	<i>Anguilla vulgaris</i> (Hamelu)	Intestine
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V. BUCCAL ORIFICE WITH THREE OR SIX LIPS

(a) Mammalia

<i>F. ascaroides</i> . Linstow	Linstow. Württemb. Naturw. Jahresh., 1879, p. 332	<i>Cercopithecus mona</i>	Bronchi
<i>F. muris</i> . Gmelin	Gmelin. Syst. Nat., p. 3032	<i>Mus musculus</i> (Vienna, Galicia, Breslau, Griefswald, Berlin, Rennes); <i>Mus decamanus</i> (Brazil)	Stomach
Syn. <i>Ascaris muris</i> <i>Fusaria muris</i> <i>Filaria obtusa</i>	Zeder. Naturg. d. Eingw., 1803, p. 106 Schneider. Monogr. d. Nemat., 1866, p. 97 Linstow. Arch. f. Naturg., xlix, 1883, p. 286 Kowalewski. Sitzber. Akad. Krakau, xxi, 1896, p. 257 Rudolphi. Entoz. Synops., 1819, pp. 27 and 249 Bremsen. Vers. intest. de l'hom., 1824, p. 126 Bremsen. Icon. Helminth., 1824 Lamarck. Anim. s. Vert., iii, 1840, p. 661 Dujardin. Hist. Nat. d. Helm., 1845, p. 89 Diesing. Syst. Helm., ii, 1851, p. 214 Baird. Catal. of Entoz. London, 1853, p. 9 Molin. Wien. Sitzber., xxxviii, 1859, p. 934 Parona. Elmintol. italiana. Genova, 1894, p. 246		
<i>Spiroptera obtusa</i>			
<i>F. verrucosa</i> . Molin	Molin. Wien. Sitzber., xxxviii, 1859, p. 964	<i>Cervus dichotomus</i> (Brazil); <i>C. namby</i> ; <i>C. paludosus</i>	Between the tendons of the phalanges
Syn. <i>Spiroptera verrucosa</i>	Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 203		

(b) Aves

<i>F. obvelata</i> . Creplin	Linstow. Arch. f. Naturg., xliii, 1877, p. 174 Parona. Elmintol. Sarda. Genova, 1887, p. 88 Braun. Arch. d. Fr. d. Naturg. i. M., 1891, p. 112 Stossich. Soc. Hist. Natur. Croa., vii, 1892, p. 72 Parona. Elmintol. italiana. Genova, 1894, p. 243 Dujardin. Hist. Nat. d. Helm., 1845, p. 101 Diesing. Spst. Helm., ii, 1851, p. 231 Molin. Wien. Sitzber., xl, 1860, p. 345 Diesing. Wien. Sitzber., xlii, 1860, p. 673	<i>Larus medius</i> ; <i>L. canus</i> (Warnemünde); <i>L. fuscus</i> ; <i>L. marinus</i> ; <i>L. ridibundus</i> (Cagliari, Trieste); <i>L. argentatus</i> ; <i>L. argentatoides</i> ; <i>L. maximus</i> (Griefswald); <i>Mergus serrator</i> ; <i>Sterna risoria</i> ; <i>Totanus fuscus</i> (Hamelu); <i>T. maculatus</i> ; <i>T. hypoleucus</i> ; <i>Alca torda</i> ; <i>Ursa grylle</i>	Oesophagus and proventriculus
<i>F. phasiani picti</i> . Molin	Molin. Wien. Sitzber., xxxviii, 1859, p. 981	<i>Phasianus pictus</i> (Vienna)	Stomach wall
Syn. <i>Spiroptera phasiani picti</i>	Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 206		
<i>F. tulostoma</i> . Hempr. et Ehr.	Schneider. Monogr. d. Nemat., 1866, p. 102	<i>Neophron percnopterus</i>	Thorax
<i>F. vulturis</i> . Molin	Molin. Wien. Sitzber., xxxviii, 1859, p. 976	<i>Cathartes papa</i> (Brazil)	Between the muscles of the lower jaw
Syn. <i>Spiroptera vulturis</i>	Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 205		
<i>F. anolabiata</i> . Molin	Molin. Wien. Sitzber., xxxviii, 1859, p. 981	<i>Crax fasciolata</i> (Brazil)	Under the nictitating membrane
Syn. <i>Spiroptera anolabiata</i>	Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 206		
<i>F. leptoptera</i> . Rudolphi	Schneider. Monogr. d. Nemat., 1866, p. 97 Linstow. Arch. f. Naturg., xliii, 1877, p. 10 Linstow. Württemb. Naturw. Jahresh., 1879, p. 325 Kowalewski. Sitzber. Akad. Krakau, xxi, 1896, p. 256	<i>Milvus regalis</i> ; <i>M. ater</i> (Griefswald); <i>Accipiter nioxus</i> (Hamelu, Ireland); <i>Circus cineraceus</i> (Vienna);	

NAME	LITERATURE	HOST	SITE
Syn. <i>Spiroptera leptoptera</i>	Rudolphi. Entoz. Synops., 1819, pp. 26 and 247 Dujardin. Hist. Nat. d. Helm., 1845, p. 93 Diesing. Syst. Helm., ii, 1851, p. 217 Baird. Catal. of Entoz. London, 1853, p. 10 Molin. Wien. Sitzber., xxxviii, 1859, p. 953	<i>C. cyaneus</i> ; <i>C. rufus</i> ; <i>Buteo vulgaris</i> (Vienna, Galicia, Berlin, Rennes); <i>Astur palumbarius</i> ; <i>Herpetotheres cachinans</i> (Brazil); <i>Harpogus bidentatus</i> (Brazil); <i>Falco tinnunculus</i> (Vienna); <i>F. lanarius</i> ; <i>F. albicollis</i> ; <i>F. subbuteo</i> (Vienna); <i>F. aurantius</i> (Brazil); <i>F. magnirostris</i> (Brazil); <i>F. xanthothorax</i> (Brazil); <i>F. uncinatus</i> (Brazil); <i>F. tridentatus</i> (Brazil); <i>Emberiza pecoris</i> (Paris)	Oesophagus, stomach and intestine
<i>F. guttata</i> . Schneider <i>F. attenuata</i> . Rudolphi	Schneider. Monogr. d. Nema., 1866, p. 92 Rudolphi. Entoz. Synops., 1819, pp. 4 and 208 Bremer. Vers. intest. d. l'hom., 1824, p. 123 Bremer. Icon. Helminth., 1824 Blanchard. Ann. d. Sc. Nat., ser. iii, tom., xi, p. 156 Lamark. Anim. s. Vert., iii, 1840, p. 667 Ecker. Arch. f. Anat. u. Phys., 1845, p. 503 Dujardin. Hist. Nat. d. Helm., 1845, p. 50 Diesing. Syst. Helm., ii, 1851, p. 266 Baird. Catal. of Entoz. London, 1853, p. 6 Wedl. Wien. Sitzber., xvii, 1855, p. 308, xix, 1856, p. 57 Leidy. Proc. Acad. Nat. Sc. Philadelph., viii, 1856, p. 56 Molin. Wien. Sitzber., xviii, 1858, p. 394; and xxx, 1858, p. 155 Diesing. Wien. Sitzber., xlii, 1860, p. 702 Molin. Denkschr. Wien. Akad., xix, 1861, p. 316 Diesing. Wien. Sitzber., xliii, 1861, p. 280 Schneider. Monogr. d. Nemat., 1866, p. 89 Stossich. Soc. Hist. Nat. Croat., vi, 1891, p. 217 Linstow. Arch. f. Naturg., 1891, p. 292 Gmelin. Syst. Nat., p. 3040 Zeder. Naturg. d. Eingw., 1803, p. 38 Molin. Wien. Sitzber., xxviii, 1858, p. 402 Diesing. Wien. Sitzber., xlii, 1860, p. 703 Molin. Wien. Sitzber., xxviii, 1858, p. 407 Diesing. Wien. Sitzber., xlii, 1860, p. 703 Molin. Wien. Sitzber., xxviii, 1858, p. 422	<i>Falco borigera</i> (Adelaide) <i>Falco subbuteo</i> , <i>F. lanarius</i> , <i>F. peregrinus</i> , <i>Otis brachyotus</i> , <i>Strix torquata</i> (Brazil) <i>Garrulus glandarius</i> (Venice)	Under the palpebral conjunctiva Thoracic and abdominal cavities and in the muscles
Syn. <i>F. falconis</i>			
<i>F. nodispina</i>			
<i>F. quadripens</i>			
<i>F. strigis torquatae</i>			

(c) Pisces

<i>F. ochracea</i> . Linstow.	Linstow. Jenaische Zeitsch., xxviii, 1893, p. 339	<i>Thymallus vulgaris</i> (Göttingen)	Stomach
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VI. OTHER FORMS

(a) Mammalia

<i>F. restiformis</i> . Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1880, p. 130	<i>Homo sapiens</i>	See chapter ii
<i>F. diacantha</i> . Molin	Railliet. Zool. méd. et agric. Paris, 1893, p. 530 Molin. Zool. méd. et agric. Paris, 1893, p. 381	<i>Cercolabes prehensilis</i> , <i>Mesomys spinosus</i> (Brazil)	Abdominal cavity and lungs
<i>F. felis mellivora</i> . Molin	Molin. Zool. méd. et agric. Paris, 1893, p. 421	<i>Felis mellivora</i> (Brazil)	Lungs
<i>F. felis onçæ</i> . Molin	Molin. Zool. méd. et agric. Paris, 1893, p. 421	<i>Felis onça</i> (Brazil)	Between the muscles
<i>F. filiformis</i> . Molin	Molin. Zool. méd. et agric. Paris, 1893, p. 396 Diesing. Zool. méd. et agric. Paris, xlii, 1860, p. 702	<i>Anabates ruffrons</i> (Brazil)	Abdominal cavity
<i>F. striata</i> . Molin	Molin. Wien. Sitzber., 1858, p. 388	<i>Felis concolor</i>	Subcutaneous
Syn. <i>Solenonema striatum</i>	Diesing. Wien. Sitzber., xlii, 1860, p. 705	<i>F. macroura</i> (Brazil)	
<i>F. scapiceps</i> . Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1886, p. 308	<i>Lepus sylvaticus</i> (N. America)	
<i>F. serpicula</i> . Molin	Molin. Proc. Acad. Nat. Sc., Philadelphia, xxviii, 1858, p. 385 Diesing. Proc. Acad. Nat. Sc., Philadelphia, xlii, 1860, p. 705	<i>Corallia brevicaudum</i> <i>Phyllostoma spiculatum</i> (Brazil)	Abdominal cavity
Syn. <i>Solenonema serpiculum</i>			
<i>F. spirocauda</i> . Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1858, p. 112 Diesing. Wien. Sitzber., xlii, 1860, p. 701 Braun. Arch. d. F. d. Naturg., i, M. 1891, p. 112 Joly. Compt. rend. Acad. d. sc., xlvii, 1856, p. 403 Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1886, p. 309	<i>Phoca vitulina</i> (Pennsylvania, Warnemünde)	Heart
Syn. <i>F. cordis phocæ</i> <i>F. stigmatura</i> . Leidy			

NAME	LITERATURE	HOST	SITE
<i>F. conica</i> . Molin	Molin. Proc. Acad. Nat. Sc., Philadelphia, xxviii, 1858, p. 412	<i>Dasyprocta aguti</i> , <i>Cavia acuschy</i> (Brazil)	Abdominal cavity
Syn. <i>Dicheilonema conicum</i>	Diesing. Proc. Acad. Nat. Sc., Philadelphia, xlii, 1860, p. 708		
<i>F. canis brachyuri</i> . Molin	Molin. Proc. Acad. Nat. Sc., Philadelphia, xxviii, 1858, p. 420	<i>Canis brachyuris</i> (Brazil)	Under the tracheal epithelium
<i>F. caprae</i> . Linstow	Linstow. Arch. f. Naturg., xlix, 1883, p. 287 Linstow. Verm. Mosca., 1886, p. 12 Railliet. Zool. medic. et agric. Paris, 1893, p. 531	<i>Capra hircus</i> (Turkestan)	Muscles under the tongue
<i>F. laevis</i> . Creplin	Creplin. System. Helm., ii, 1851, p. 265 Molin. Wien. Sitzber, xxviii, 1858, p. 389 Diesing. Wien. Sitzber, xlii, 1860, p. 701	<i>Tarsius spectrum</i>	Under the skin
<i>F. leonis</i> . Gmelin	Gmelin. Syst. Nat., i, 1788, p. 3040 Rudolphi. Entoz. Synops., 1819, p. 7 Diesing. Syst. Helm., ii, 1851, p. 280 Molin. Wien. Sitzber, xxviii, 1858, p. 421	<i>Felis leo</i>	Under the skin
Syn. <i>Ascaris leonis</i>	Gmelin. Syst. Nat., i, 1788, p. 3031		
<i>F. leporis</i> . Gmelin	Gmelin. Syst. Nat., i, 1788, p. 3040 Zeder. Naturg. d. Eingw., 1803, p. 38 Rudolphi. Entoz. Synops., 1819, p. 8 Dujardin. Hist. Nat. d. Helm., 1845, p. 48 Diesing. Syst. Helm., ii, 1851, p. 280 Molin. Wien. Sitzber, xxviii, 1858, p. 421 Molin. Wien. Sitzber, xxviii, 1858, p. 401	<i>Lepus timidus</i>	Thigh and lumbar region
<i>F. bidentata</i> . Molin		<i>Cervus nambi</i> ; <i>C. simplicornis</i> ; <i>C. rufus</i> (Brazil)	Abdominal cavity
<i>F. incrassata</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 389 Diesing. Wien. Sitzber, xlii, p. 700	<i>Nasua socialis</i> <i>Bradypus tridactylus</i> (Brazil)	
<i>F. inflexo candata</i> . Siebold	Siebold. Wiegmann's Arch., 1842, p. 348 Diesing. Syst. Helm., ii, 1851, p. 281 Baird. Catal. of Entoz. London, 1853, p. 7 Molin. Wien. Sitzber, xxviii, 1858, p. 422 Benedin. Bull. Acad. Roy. Bruxelles Sc., tom. xxix, 1870, p. 364	<i>Phocaena communis</i>	Encysted in the lungs
<i>F. insignis</i> . Leidy	Leidy. Proc. Acad. Nat. Sc. Philadelphia, 1858, p. 112 Diesing. Wien. Sitzber, xlii, 1860, p. 711	<i>Procyon lotor</i>	Encysted under skin of feet
<i>F. intercostalis</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 418	<i>Chrysothrix sciurea</i> (Brazil)	Between the inter-costal muscles
<i>F. bifida</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 411	<i>Dactylomys amblyonyx</i> (Brazil)	Liver
Syn. <i>Dicheilonema bifidum</i>	Diesing. Wien. Sitzber, xlii, 1860, p. 707		
<i>F. nodosa</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 380 Diesing. Wien. Sitzber, xlii, 1860, p. 701	<i>Hapale melanura</i> ; <i>Callithrix personata</i>	Beneath the skin
<i>F. pistillaris</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 381 Diesing. Wien. Sitzber, xlii, 1860, p. 701	<i>Sciurus igniventris</i> (Brazil)	Beneath the skin
<i>F. annulata</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 386	<i>Lagothrix cana</i> (Brazil)	
<i>F. anticlava</i> . Molin	Molin. Wien. Sitzber, xxviii, p. 381 Diesing. Wien. Sitzber, xlii, 1860, p. 701	<i>Dasytus sexcintus</i>	Stomach
<i>F. acuticauda</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 379	<i>Dasytus loricatus</i> ; <i>D. niger</i> (Brazil)	Under the skin of the neck
<i>F. aequalis</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 383	<i>Myrmecophaga jubatu</i> (Brazil)	
Syn. <i>Solenonema aequale</i>	Diesing. Wien. Sitzber, xlii, 1860, p. 704	<i>Lepus timidus</i> (Tuscany)	Lungs
<i>F. terminalis</i> . Passerini	Passerini. Atti Soc. Ital. d. sc. nat. Milano, xxviii, 1884, p. 42 Parona. Elmintol. italiana. Genova, 1894, p. 241		
<i>F. oculi canini</i> . Gescheidt	Railliet. Zool. med. et agric. Paris, 1893, p. 531	<i>Canis familiaris</i>	Vitreous body of eye
Syn. <i>F. trispinalosa</i>	Diesing. Syst. Helm., ii, 1851, p. 274 Molin. Wien. Sitzber, xxviii, 1858, p. 402 Davaine. Traité d. Entoz. Paris, 1877, p. 108		
<i>F. vulpis</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 7 Diesing. Syst. Helm., ii, 1851, p. 280 Molin. Wien. Sitzber, xxviii, 1858, p. 420	<i>Canis vulpes</i>	Abdomen
<i>F. vespertilionis</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 7 Dujardin. Hist. Nat. d. Helm., 1845, p. 47 Diesing. Syst. Helm., ii, 1851, p. 279	<i>Vespertilio discolor</i> <i>V. bechsteinii</i>	Abdomen
<i>F. torta</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 419 Molin. Wien. Sitzber, xxviii, 1858, p. 390 Diesing. Wien. Sitzber, xlii, 1860, p. 700	<i>Lagothrix cana</i> (Brazil)	
<i>F. macropodis gigantis</i> . Webster	Diesing. Syst. Helm., ii, 1851, p. 280	<i>Macropus giganteus</i>	Knee
<i>F. turdi olivascens</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 422 Molin. Wien. Sitzber, xlii, 1860, p. 423	<i>Turdus olivascens</i> (Brazil)	Beneath the nectitating membrane
<i>F. dubia</i> . Stossich			
Syn. <i>F. verrucosa</i>	Molin. Wien. Sitzber, xlii, 1860, p. 392 Diesing. Wien. Sitzber, xlii, 1860, p. 702	<i>Falco swainsonii</i> (Brazil)	Between the muscles of the lower jaw
<i>F. papilloso-annulata</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 399 Diesing. Wien. Sitzber, xlii, 1860, p. 702	<i>Strix suinda</i> <i>Falco swainsonii</i> (Brazil)	Orbit

NAME	LITERATURE	HOST	SITE
(b) Aves			
<i>F. campanulata</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 392 Diesing. Wien. Sitzber, xlii, 1860, p. 702	<i>Falco magnirostris</i> (Brazil)	
<i>F. tendo</i> . Nitzsch		<i>Falco peregrinus</i>	
<i>F. labiotruncata</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 412	<i>Tinamus adspersus</i> , <i>T. strigulosus</i> , <i>T. variegatus</i> (Brazil)	Abdominal cavity, and under skin
Syn. <i>Dicheilonema labiotruncatum</i>	Diesing. Wien. Sitzber, xlii, 1860, p. 708	<i>Tinamus rufescens</i> , <i>T. maculosus</i> (Brazil)	Abdominal cavity, and subcutaneous
<i>F. quadrilabata</i> . Molin	Molin. Wien. Sitzber, xlii, 1860, p. 417		
Syn. <i>Tetracheilonema quadrilabiatum</i>	Diesing. Wien. Sitzber, xlii, 1860, p. 711		
<i>F. tinami variegati</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 427	<i>Tinamus variegatus</i> (Brazil)	Beneath the necti- tating membrane
<i>F. subspiralis</i> . Diesing	Diesing. Syst. Helm., ii, 1851, p. 268 Molin. Wien. Sitzber, xxviii, 1858, p. 391 Diesing. Wien. Sitzber, xlii, 1860, p. 700	<i>Ardea cinerea</i> , <i>A. leucogaster</i> (Brazil)	Beneath the skin of the feet
Syn. <i>F. ardeae cinereae</i>	Rudolphi. Entoz. Synops., 1819, p. 9 Dujardin. Hist. Nat. d. Helm., 1845, p. 56		
<i>F. ardeae</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 428	<i>Ardea exilis</i> (Brazil)	Under the tongue
<i>F. myotherae campanisonae</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 425	<i>Formicivora campanisona</i> (Brazil)	Eye
<i>F. myotherae chrysopygae</i> . Molin	Molin. Hist. Nat. d. Helm., 1858, p. 425	<i>Formicivora chrysopyga</i> (Brazil)	Under skin near the eyes
<i>F. myotherae regis</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 424	<i>Formicivora rex</i> (Brazil)	Kidney
<i>F. myotherae ruficipitis</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 424	<i>Formicivora ruficeps</i> (Brazil)	Abdominal cavity
<i>F. tridens</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 393 Diesing. Hist. Nat. d. Helm., xlii, 1860, p. 702	<i>Lanius collurio</i> ; <i>Icterus cristatus</i> ; <i>I. haemorrhous</i> ; <i>I. icterocephalus</i> ; <i>I. chopi</i> ; <i>I. sericeus</i> ; <i>Cassicus ater</i> ; <i>C. viridis</i>	Abdominal and thoracic cavities
Syn. <i>F. cassiciatri</i>	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 423	<i>Icterus pyrrhopterus</i> (Brazil)	Abdominal and thoracic cavities
<i>F. icteri pyrrhopteri</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, p. 423	<i>Strix suinda</i> (Brazil)	Under the skin of the neck
<i>F. bipapillosa</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 399 Diesing. Hist. Nat. d. Helm., xlii, 1860, p. 702	<i>Strix flammea</i> (Brazil)	Abdominal cavity
<i>F. hystrix</i> . Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 408 Diesing. Hist. Nat. d. Helm., xlii, 1860, p. 703		
<i>F. acuta</i> . Diesing	Diesing. Syst. Helm., ii, 1851, p. 277 Molin. Wien. Sitzber, xxviii, 1858, p. 411 Braun. Arch. d. Fr. d. Naturg., i, M., 1891, p. 112	<i>Podiceps cristatus</i> (Rostock); <i>P. cornutus</i>	Abdominal cavity
Syn. <i>F. colymbi</i>	Rudolphi. Entoz. Synops., 1819, p. 10		
<i>Dicheilonema acutum</i>	Diesing. Wien. Sitzber, xlii, 1860, p. 707	<i>Podiceps auritus</i> (Caen)	Abdominal cavity
<i>F. subulata</i> . Deslongchamps	Dujardin. Hist. Nat. d. Helm., 1845, p. 58 Diesing. Syst. Helm., ii, 1851, p. 283		
<i>F. clavato-verrucosa</i> . Molin	Molin. Wien. Sitzber, xxviii, p. 429	<i>Thamnophilus canadensis</i> (Brazil)	On the intestine
<i>F. attenuato-verrucosa</i> . Molin	Molin. Wien. Sitzber, xxviii, p. 380 Diesing. Wien. Sitzber, xlii, 1860, p. 701	<i>Thamnophilus canadensis</i> (Brazil)	Internal body cavity
<i>F. piprae caudatae</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 394 Diesing. Wien. Sitzber, xlii, 1860, p. 702	<i>Pipra caudata</i> (Brazil)	Abdominal cavity
<i>F. tricornonata</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 424 Diesing. Wien. Sitzber, xlii, 1860, p. 702	<i>Pipra inornata</i> (Brazil)	Abdominal cavity
<i>F. veneta</i> . Stossich			
Syn. <i>F. quadrispina</i>	Molin. Wien. Sitzber, xxxiii, 1858, p. 301 Molin. Denkschr. Wien. Akad., xix, 1861, p. 318 Diesing. Wien. Sitzber, xliii, 1861, p. 280 Parona. Elmintol. italiana. Genova, p. 243	<i>Ibis falcinellus</i> (Padua)	Between the walls of the stomach
<i>F. tantali cayennensis</i> . Molin	Molin. Elmintol. italiana. Genova, p. 435	<i>Ibis cayennensis</i> (Brazil)	Stomach wall
<i>F. carduelis</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 9 Dujardin. Hist. Nat. d. Helm., 1845, p. 54 Diesing. Syst. Helm., ii, 1851, p. 281	<i>Fringilla carduelis</i>	Thigh
<i>F. affinis</i> . Rudolphi	Molin. Wien. Sitzber, xxviii, 1858, p. 423 Rudolphi. Entoz. Synops., 1819, pp. 4 and 209 Dujardin. Hist. Nat. d. Helm., 1845, p. 54 Diesing. Syst. Helm., ii, 1851, p. 268	<i>Fringilla hispaniolensis</i> (Spain)	Abdominal cavity
<i>F. dendrocalaptes procurvi</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 396 Diesing. Wien. Sitzber, xlii, 1860, p. 702	<i>Dendrocalaptes procurvus</i> (Brazil)	Eye
<i>F. quadriverrucosa</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 398 Diesing. Wien. Sitzber, xlii, 1860, p. 702	<i>Dendrocalaptes picus</i> ; <i>D. rufirostris</i> (Brazil)	Abdominal cavity
<i>F. coronata</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 6 Lamark. Anim. s. Vert., iii, 1840, p. 668 Dujardin. Hist. Nat. d. Helm., 1845, p. 55 Diesing. Syst. Helm., ii, 1851, p. 275	<i>Coracias garrula</i> (Padua, Vienna, Turkestan)	Under the muscles of head and skin of neck
	Molin. Wien. Sitzber, xxviii, 1858, p. 408, and xxx, 1858, p. 155 Diesing. Wien. Sitzber, xlii, 1860, p. 703; xliii, 1861, p. 280		

NAME	LITERATURE	HOST	SITE
<i>F. coronata</i> . Rudolphi—contd.	Linstow. Arch. f. Naturg., xlix, 1883, p. 286 ; Vermi, Mosca, 1886, p. 11 Parona. Elmintol italiana. Genova, 1894, p. 242 Gmelin. Syst. Nat., p. 3033 Zeder. Naturg. d. Eingw., 1803, p. 119		
Syn. <i>Ascaris coraciae</i> <i>Fusaria coraciae</i>	Leidy. Proc. Acad. Nat. Sc. Philadelphia, 1886, p. 308 Leidy. Proc. Acad. Nat. Sc. Philadelphia, 1885, p. 10	<i>Funco hyemalis</i> <i>Sturnella magna</i> <i>Sturnus vulgaris</i>	Lungs and thoracic cavity
<i>F. obtusa</i> . Leidy. Syn. <i>F. obtusa</i> <i>F. sturni</i> . Rudolphi.	Rudolphi. Entoz. Synops., 1819, p. 9 Dujardin. Hist. Nat. d. Helm., 1845, p. 53 Diesing. Syst. Helm., ii, 1851, p. 281 Molin. Wien. Sitzber, xxviii, 1858, p. 424		
<i>F. abbreviata</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, pp. 4 and 210 Dujardin. Hist. Nat. d. Helm., 1845, p. 52 Diesing. Syst. Helm., ii, 1851, p. 268 Molin. Wien. Sitzber, xxviii, 1858, p. 396 Linstow. Arch. f. Naturg., xlix, 1883, p. 286 Linstow. Vermi. Mosca, 1886, p. 11 Rudolphi. Entoz. Synops., 1819, p. 635 Rudolphi. Entoz. Synops., 1819, p. 9 Rudolphi. Entoz. Synops., 1819, p. 9 Diesing. Syst. Helm., ii, 1851, p. 226	<i>Saxicola sp.</i> (Turkestan) ; <i>Luscinia philomela</i> ; <i>Motacilla melanocephala</i> (Brazil) ; <i>Saxicola oenanthe</i> ; <i>S. stapezina</i> ; <i>Luscinia rubecula</i> ; <i>Turdus pilaris</i> ; <i>T. viscivorus</i> ; <i>Sturnus pyrrhocephalus</i> (Brazil) ; <i>Tanagra jacapa</i> (Brazil) ; <i>Thryothorus polyglottus</i> (Brazil) ; <i>Furnarius rufus</i> (Brazil) ; <i>F. leucops</i> (Brazil)	The internal cavities of the body
Syn. <i>F. motacillae</i> <i>F. motacillarum</i> <i>F. turdorum</i> <i>F. philomelae</i>		<i>Psittacus makaonanna</i> (Brazil)	Beneath the skin
<i>F. simplicissima</i> . Molin	Molin. Syst. Helm., xxviii, 1858, p. 372 Diesing. Syst. Helm., xlii, 1860, p. 701 Dujardin. Hist. Nat. d. Helm., 1845, p. 56 Diesing. Syst. Helm., ii, 1851, p. 283 Molin. Wien. Sitzber, xxviii, 1858, p. 427 Linstow. Arch. f. Naturg., 1883, xlix, p. 287 Linstow. Vermi. Mosca, 1886, p. 11	<i>Vanellus cristatus</i> (Caen, Turkestan)	Abdominal cavity
<i>F. truncato-caudata</i> . Deslongchamps	Molin. Wien. Sitzber, xviii, 1858, p. 400 Diesing. Syst. Helm., ii, 1851, p. 277 Molin. Wien. Sitzber, xxviii, 1858, p. 411 Rudolphi. Entoz. Synops., 1819, p. 10 Diesing. Wien. Sitzber, xlii, 1860, p. 707	<i>Muscicapa sp.</i> (Brazil) <i>Sterna leucopareia</i>	Abdomen Abdominal cavity
<i>F. bifurca</i> . Molin <i>F. bilabiate</i> . Diesing	Molin. Wien. Sitzber, xviii, 1858, p. 400 Diesing. Syst. Helm., ii, 1851, p. 277 Molin. Wien. Sitzber, xxviii, 1858, p. 411 Rudolphi. Entoz. Synops., 1819, p. 10 Diesing. Wien. Sitzber, xlii, 1860, p. 707		
Syn. <i>F. sternae</i> <i>Dicheilonema bilabiatum</i> <i>F. cyngi</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 10 Dujardin. Hist. Nat. d. Helm., 1845, p. 58 Diesing. Syst. Helm., ii, 1851, p. 284 Molin. Wien. Sitzber, xxviii, 1858, p. 429 Railliet. Zool. med. et Agric. Paris, 1893, p. 533 Parona. Elmintol. italiana. Genova, 1894, p. 243 Gmelin. Syst. Nat., p. 3033 Zeder. Naturg. d. Eingw., 1803, p. 119 Diesing. Syst. Helm., ii, 1851, p. 275 Molin. Wien. Sitzber, xxviii, 1858, p. 404 Diesing. Wien. Sitzber, xlii, 1860, p. 703	<i>Cygnus musicus</i>	Abdominal cavity
<i>F. anatis</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 20 Dujardin. Hist. Nat. d. Helm., 1845, p. 58 Diesing. Syst. Helm., ii, 1851, p. 284 Molin. Wien. Sitzber, xxviii, 1858, p. 429 Railliet. Zool. med. et agric. Paris, 1893, p. 533 Nordmann. Microgr. Beiträge, i, 1832, p. 17 Diesing. Syst. Helm., ii, 1851, p. 281 Molin. Wien. Sitzber, xxviii, 1858, p. 423 Molin. Wien. Sitzber, xxviii, 1858, p. 401	<i>Buteo lagopus</i> <i>Anas domestica</i>	In vitreous humour of the eye The leg
<i>F. sylviae</i> . Nordmann		<i>Sylvia abietina</i>	Orbit
<i>F. spaerophora</i> . Molin		<i>Anabates anthoides</i> ; <i>Muscicapa lophotes</i> (Brazil) <i>Glareola austriaca</i>	Liver Stomach wall
<i>F. spinulosa</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 350 Diesing. Wien. Sitzber, xlii, 1860, p. 712 Molin. Wien. Sitzber, xxviii, 1858, p. 426 Rudolphi. Entoz. Synops., 1819, pp. 10 and 218 Dujardin. Hist. Nat. d. Helm., 1845, p. 58 Diesing. Syst. Helm., ii, 1851, p. 283 Molin. Wien. Sitzber, xxviii, 1858, p. 429	<i>Calliphlox amethystina</i> (Brazil) <i>Larus minutus</i> (Vienna)	On the stomach Under the skin of the neck
<i>F. trochili amethystini</i> . Molin <i>F. lari</i> . Rudolphi		<i>Merops apiaster</i>	Mesentery
<i>F. meropis</i> . M.C.V.	Rudolphi. Entoz. Synops., 1819, p. 9 Dujardin. Hist. Nat. d. Helm., 1845, p. 55 Diesing. Syst. Helm., ii, 1851, p. 281 Molin. Wien. Sitzber, xxviii, 1858, p. 426	<i>Otis brachyotus</i> <i>Podoa surinamensis</i> (Brazil) <i>Charadrius fluviatilis</i> (Vienna)	Subcutaneous Beneath the skin of the neck Under the skin of the nose and ear
<i>F. aspera</i> . Nitzsch <i>F. podae</i> . Molin	Molin. Wien. Sitzber, xxviii, 1818, p. 428		
<i>F. charadru</i> . M.C.V.	Rudolphi. Entoz. Synops., 1819, p. 10 Dujardin. Hist. Nat. d. Helm., 1845, p. 56 Diesing. Syst. Helm., ii, 1851, p. 283 Molin. Wien. Sitzber, xxviii, 1858, p. 427 Molin. Wien. Sitzber, xxviii, 1858, p. 377	<i>Trogon aurantius</i> (Brazil)	Abdominal cavity
<i>F. circumflexa</i> . Molin			

NAME	LITERATURE	HOST	SITE
<i>F. tringae</i> . M.C.V.	Rudolphi. Entoz. Synops., 1819, p. 10	<i>Tringa variabilis</i>	Beneath the skin
<i>F. serotina</i> . Molin	Diesing. Syst. Helm., 1851, p. 282	<i>Lichenops perspicillata</i> (Brazil)	Abdominal cavity
<i>F. fusiformis</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 374	<i>Monasa tranquilla</i> (Brazil)	Thoracic cavity
Syn. <i>Dicheilonema fusiforme</i>	Diesing. Wien. Sitzber, xlii, 1860, p. 701	<i>Alcedo amazona</i> ; <i>A. torquati</i> ,	Abdominal cavity
<i>F. physalura</i> . Bremser	Molin. Wien. Sitzber, xlii, 1860, p. 709	<i>A. superciliosa</i> ; <i>Ceryle alcyon</i>	(Brazil)
Syn. <i>F. alcedinus</i>	Diesing. Syst. Helm., ii, 1851, p. 256	<i>Corvus torquati</i>	Right ventricle of heart and pulmonary
<i>F. alcedinus superciliosae</i>	Molin. Wien. Sitzber, xxviii, 1858, p. 412	<i>Pica media</i>	In tubercles on the pulmonary and aortic valves
<i>Monopetalonema physalurum</i>	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1885, p. 10	<i>Plotus anHINGA</i> (Florida)	Brain
<i>F. corvi torquati</i> . Cobbold and Manson	Rudolphi. Entoz. Synops., 1819, p. 635	<i>Pionus menstruus</i> (Brazil)	Under the skin of the neck
<i>F. picae mediae</i> . Cobbold and Manson	Molin. Wien. Sitzber, xlii, 1860, p. 710	<i>Tetrao bonasio</i>	Eye
<i>F. helicina</i> . Molin.	Molin. Wien. Sitzber, xxviii, 1858, p. 391	<i>Perdix dentata</i> (Brazil)	Abdominal cavity
<i>F. hemicycla</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 377	<i>Botaurus minor</i> (America)	Proventricule
<i>F. bonasiae</i> . Nordmann	Diesing. Wien. Sitzber, xlii, 1860, p. 701	<i>Platyrrhynchus petangua</i> (Brazil)	Abdominal cavity
<i>F. perdicis dentatae</i> . Molin.	Dujardin. Hist. Nat. d. Helm., 1845, p. 56	<i>Guiscaulus major</i> (Florida)	
<i>F. triaenucha</i> . Wright	Diesing. Syst. Helm., ii, 1851, p. 282		
<i>F. dipetala</i> . Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 426		
Syn. <i>Dipeta lonema inflexum</i>	Molin. Wien. Sitzber, xxviii, 1858, p. 427		
<i>F. cirrura</i> . Leidy	Wright. Americ. Helminth. I, 1879, p. 21		
	Molin. Wien. Sitzber, xxviii, 1858, p. 373		
	Diesing. Wien. Sitzber, xlii, 1860, p. 704		
	Leidy. Proc. Acad. Nat. Sc. Philadelph. viii, 1886, p. 309		

(c) Reptilia

<i>F. mucronata</i> . Molin	Molin. Wien. Sitzber, xxx, 1858, p. 155		
Syn. <i>Dipetalonema mucronatum</i>	Molin. Denkschr. Wien. Akad., xix, 1861, p. 318	<i>Boa constrictor</i>	Thoracic cavity
<i>F. bispinosa</i> . Diesing	Diesing. Wien. Sitzber, xlii, 1860, p. 704, and xliii, 1861, p. 280	<i>Boa constrictor</i> ; <i>Ophis saurocephalus</i> ; <i>Thamnobius poecitostomo</i> (Brazil)	Under the skin, in the abdominal cavity, and in the walls of the oesophagus and intestine
Syn. <i>Dicheilonema bispinosum</i>	Diesing. Syst. Helm., ii, 1851, p. 278		
<i>F. boae constrictoris</i>	Leidy. Proc. Acad. Nat. Sc. Philadelphia, viii, 1856, p. 56	<i>Coronella austriaca</i>	Oesophagus
<i>F. megalochila</i> . Diesing	Diesing. Denkschr. Wien. Akad., xiii, 1879, p. 18		
Syn. <i>F. colubri anstriaci</i>	Molin. Wien. Sitzber, xxviii, 1858, p. 415		
<i>Tricheilonema megalochilum</i>	Diesing. Wien. Sitzber, xlii, 1860, p. 709		
<i>F. podinemae scriptae</i> . Molin	Leidy. Proc. Acad. Nat. Sc. Philadelphia, v, 1851, p. 118	<i>Podinema scripta</i> (Brazil)	In the abdominal fat
<i>F. haje</i> . Wedl.	Diesing. Syst. Helm., ii, 1851, p. 278	<i>Naja haje</i>	All parts external to lungs
<i>F. hebetata</i> . Cobbold	Molin. Wien. Sitzber, xxviii, 1858, p. 417	<i>Cystophora cristata</i>	Right heart
<i>Filaria bacillaris</i> . Molin	Rudolphi. Entoz. Synops., 1819, p. 10	<i>Caiman niger</i> ; <i>C. sclerops</i> (Brazil)	Lungs
<i>F. cloeliae fasciatae</i> . Molin	Diesing. Wien. Sitzber, xlii, 1860, p. 711	<i>Oxyrhopus fasciatis</i> (Brazil)	Encysted in stomach wall
<i>F. colubri</i> . Box	Molin. Wien. Sitzber, xxviii, 1858, p. 430	<i>Coluber sp.</i> (America)	Intestine
Syn. <i>F. colubri americani</i>	Box. Hist. Nat. d. vers. Paris, ii, 1802, p. 49		
<i>F. colubri aenei</i> . Molin	Dujardin. Hist. nat. d. Helm., 1845, p. 58	<i>Helicops carinicaudus</i>	Abdominal cavity
<i>F. multipapilla</i> . Molin	Diesing. Syst. Helm., ii, 1851, p. 284	<i>Thorictis dracaena</i> ; <i>Iguana tuberculata</i> (Brazil)	Abdominal cavity
<i>F. calcarata</i> . Molin.	Molin. Wien. Sitzber, xxviii, 1858, p. 431	<i>Bothrops jararacca</i> (Brazil)	Abdominal cavity
<i>F. eunectis scytalis</i> . Molin	Rudolphi. Entoz. Synops., 1819, p. 10	<i>Eunectes scytale</i> (Brazil)	Lungs
<i>F. solitaria</i> . Leidy	Molin. Wien. Sitzber, xxviii, 1858, p. 435	<i>Emys serrata</i> ; <i>Chelonura serpentina</i> (Georgia)	Wall of stomach
<i>F. cistudinis</i> . Leidy	Molin. Wien. Sitzber, xxviii, 1858, p. 385		
	Diesing. Wien. Sitzber, xlii, 1860, p. 700		
	Molin. Wien. Sitzber, xxviii, 1858, p. 378		
	Diesing. Wien. Sitzber, xlii, 1860, p. 701		
	Molin. Wien. Sitzber, xxviii, 1858, p. 430		
	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56		
	Molin. Wien. Sitzber, xxviii, 1858, p. 430		
	Diesing. Wien. Sitzber, xlii, 1860, p. 702		
	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56		
	Molin. Wien. Sitzber, xxviii, 1858, p. 430		

(d) Amphibia

NAME	LITERATURE	HOST	SITE
<i>F. rubella</i> . Rudolphi	Rudolphi. Entoz. Synops., 1819, pp. 5 and 212 Dujardin. Hist. Nat. d. Helm., 1845, p. 59 Diesing. Syst. Helm., ii, 1851, p. 269 Molin. Wien. Sitzber., xxviii, 1858, p. 372	<i>Rana esculenta</i> (Berlin)	Mesentery; stomach and intestinal walls
Syn. <i>F. ranæ esculentæ</i>	Valentin. Wiegmann's Arch., 1842, p. 312 Diesing. Syst. Helm., ii, 1851, p. 284 Molin. Wien. Sitzber., xxviii, 1858, p. 431		
<i>F. neglecta</i> . Diesing	Diesing. Syst. Helm., ii, 1851, p. 276 Molin. Wien. Sitzber., xxviii, 1858, p. 409 Diesing. Wien. Sitzber., xlii, 1860, p. 703 Rudolphi. Entoz. Synops., 1819, p. 10	<i>Rana esculenta</i>	Under the skin
Syn. <i>ranæ esculentæ</i>			
<i>F. nitida</i> . Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber., xxviii, 1858, p. 378	<i>Rana pipiens</i> (America)	Encysted on the peritoneum and abdominal muscles
<i>F. convoluta</i> . Molin	Molin. Wien. Sitzber., xxviii, 1858, p. 390 Diesing. Wien. Sitzber., xlii, 1860, p. 702	<i>Cystignatus gigas</i> , <i>Leptidactylus sibilatrix</i> (Brazil)	Abdomen
<i>F. amphiumæ</i> . Leidy	Leidy. Proc. Acad. Nat. d. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber., xxviii, 1858, p. 431	<i>Amphiuma means</i> (Philadelphia)	In stomach wall
<i>F. eupemphigis marmorati</i> . Molin	Molin. Wien. Sitzber., xxviii, 1858, p. 431	<i>Eupemphix marmoratus</i> (Brazil)	Abdominal cavity

(e) Pisces

<i>F. triglae</i> . Bellingham	Bellingham. Ann. of Nat. Hist., xiv, 1844, p. 475 Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber., xxviii, 1858, p. 432	<i>Trigla cuculus</i> (Ireland)	Peritoneum
<i>F. quadrituberculata</i> . Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber., xxviii, 1858, p. 410 Bellingham. Ann. of Nat. Hist., xiv, 1844, p. 475	<i>Anguilla vulgaris</i> (America)	Dorsal muscles
<i>F. mugilis</i> . Bellingham	Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber., xxviii, 1858, p. 433 Molin. Wien. Sitzber., xxviii, 1858, p. 431	<i>Mugil capito</i> (Ireland)	Peritoneum
<i>F. ranæ</i> . M.C.V.	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber., xxviii, 1858, p. 415 Diesing. Wien. Sitzber., xlii, 1860, p. 708	<i>Hypsiboas faber</i> (Brazil)	Intestine
<i>F. rubra</i> . Leidy	Nordmann. Microgr. Beiträge, 1832, p. 20 Dujardin. Hist. Nat. d. Helm., 1845, p. 62 Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber., xxviii, 1858, p. 433	<i>Labrax lineatus</i> (America)	Peritoneum
Syn. <i>Dicheilonea rubrum</i>			
<i>F. crassiuscula</i> . Nordmann.	Nordmann. Microgr. Beiträge, 1832, p. 20 Dujardin. Hist. Nat. d. Helm., 1845, p. 62 Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber., xxviii, 1858, p. 433	<i>Gadus aeglefinis</i>	Eye
<i>F. extenuata</i> . Deslongchamps	Dujardin. Hist. Nat. d. Helm., 1845, p. 61 Diesing. Syst. Helm., ii, 1851, p. 285 Molin. Wien. Sitzber., xxviii, 1858, p. 432	<i>Mullus surmuletus</i> (Caen)	Abdomen

(f) Coelenterata

<i>F. loliginis</i> . Delle Chiaje	Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber., xxviii, 1858, p. 434 Parona. Elmintol. italiana. Genova, 1894, p. 244	<i>Loligo vulgaris</i> (Naples)	
<i>F. succineæ</i> . Siebold	Siebold. Wiegmann's Arch., 1837, p. 255 Diesing. Syst. Helm., ii, 1851, p. 287 Molin. Wien. Sitzber., xxviii, 1858, p. 434	<i>Succinea amphibia</i>	Abdomen

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<i>F. bancroftii</i> . Cobbold	Railliet. Zool. médic. et agric. Paris, 1895, p. 515 Ward. The paras. worms of man and the dom. anim., 1894, p. 390 De Bonis. I. paras. d. corpo umano. Naples, 1876, p. 130 Davaine. Traité d. Entoz. Paris, 1877, p. 107 Küchenmeister et Zürn. Paras. d. Mensch., 1881, p. 431 Manson. Trans. Linn. Soc. London, 1884, April Sonsino. Pr. verb. Soc. toscana di sc. nat., 1884, July Jaksch, R.v. Klin. Diagn. inn. Krankh., 1889, p. 43 Blanchard. Anim. par. introd. par l'eau. Paris, 1890, p. 74 Huber. Bibliograph. d. Klin. Helminth. München, 1894, p. 268 Sonsino. Mem. de l'Institut. égyptien. Cairo, 1896, p. 320 Magalhães. Revista de Cursos prat. e theor. d. Fac. d. medic. do Rio de Janeiro, iii, 1887, p. 129 Maitland. Lancet, 1897, ii, p. 1483 Manson. Brit. Med. Journ., 1896, ii, p. 1379 Thorpe. Brit. Med. Journ., 1896, ii, p. 922 Firkett. Bull. Acad. roy. de med. de Belg. Bruxelles, 1895, 4 s., ix, p. 669-685 Manson. Brit. Med. Journ., 1893, i, p. 792 Manson. Tr. vii Intern. Cong. Hyg. and Demog. London, 1891, i, p. 79
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THE HIBERNATION OF MOSQUITOES

THE HIBERNATION OF ENGLISH MOSQUITOES*

BY

H. E. ANNETT, M.D., AND J. EVERETT DUTTON, M.B.

In the *British Medical Journal* of April 27, 1901, Dr. M. J. WRIGHT records some interesting experiments concerning the resistance of mosquito larvae to cold. It is truly a remarkable feature that larvae, both *Culex* and *Anopheles*, are able to withstand a temperature of about 4° C. for a period of two weeks.

Early in December last Mr. T. V. THEOBALD, of Wye, Kent, sent a number of *Anopheles* larvae to Major Ross, which have been kept continuously in a greenhouse at a temperature of from 15° C. to 32° C., and, although they are often seen feeding on the green protococcal growth supplied, they show apparently very little increase in size, and none have as yet changed † into pupae.

Dr. WRIGHT infers from his observations that the larval form is that in which 'hibernation' takes place; never having found adult mosquitoes during the winter months. Here his results differ most markedly from ours, which we wish now to record in a short preliminary account. It may be mentioned that throughout the winter session a supply of *Culex* adults has been obtainable for class purposes at this School.

On February 17, during a period of very cold weather, four *Anopheles* were caught at a farm some thirteen miles from Liverpool, in North Cheshire. A large number of *Culex* (four species) were also captured here. The *Anopheles* were identified by Mr. T. V. THEOBALD as *A. maculipennis*. The mosquitoes were found in the following situations: cellar, dairy, cheese room, pantries, lumber rooms, and in some disused bedrooms at the top of the house; also in the wash-house and whey tank house abutting on to the house, and in the coach-house, tool sheds, and privies at some distance away. No mosquitoes could be found in the stables, cowsheds, pigsties, haylofts, henpens. Many of the farm houses of this district of Cheshire are old, and have no damp-proof courses. In the disused cellars the walls and the beams supporting the ground floor were soaking with moisture, and small ferns grew in the crevices of the tiled floor, and patches of moss and mould on the surface of the walls. Here thousands of mosquitoes, chiefly *Culex*, blackened the walls and rafters. In the dairies they were found on the damp areas, resting on and in the crevices of the plaster; very few were seen on the drier parts. Similarly in the other places, on the damp portions, many mosquitoes were observed, especially behind boxes, slates, boards, barrels, and other articles resting against the wall.

* The greater part of this article appeared in the *British Medical Journal*, April 27, p. 1013, 1901.

† April 16, 1901.

Since the date* mentioned, *Anopheles* have been collected on four occasions from farms in North and Mid Cheshire. The Mid Cheshire farm is at a distance of about thirty-five miles from Liverpool, and here *Anopheles maculipennis* occurred in about the same number and in similar situations as in the North Cheshire farms. About twenty *Anopheles* were collected at each farm. In all these sites it was noted that the majority of the mosquitoes of the genus *Culex* were found on the parts of the damp walls near the ground, while *Anopheles* were generally caught near the ceiling. During the coldest weather the attitude both of *Culex* and *Anopheles* was peculiar and characteristic. The under surface of the thorax and abdomen was applied closely to the wall, while the legs were stretched straight out almost at right angles to the body. The absence of the characteristic attitude of *Anopheles* (at an angle to the surface), and the fact that both *Culex* and *Anopheles* assumed the peculiar outstretched attitude, made it difficult at first sight to distinguish specimens of the two genera, especially since among the *Culex* were a species having wings spotted somewhat similarly to *Anopheles* (*Culex annulatus*): but on closer inspection even in the position described, the characteristic angle, seen in side view, between the direction of the head and thorax and of the abdomen of *Culex*, served to distinguish the genera. In this peculiar attitude the mosquitoes were very difficult to rouse; the mouth of a bottle could be easily placed over them without disturbing them, and in fact, one had to lift them on to their legs by the rim, and then no attempt was made to fly: they would crawl lazily along the neck of the bottle. How long these mosquitoes remain in this position during the winter months is not easy to determine, but it was noticed that many of the *Culex* on the damper patches were wholly or partially enveloped in a thick mould which had grown in and around their bodies, thus fixing them in the attitude described. On very cold days this attitude was observed even in the bottles in which the mosquitoes had been collected. On warmer days at the farms, and on taking the bottled mosquitoes into a warm room, they assumed their ordinary attitudes.

It has been mentioned above that mosquitoes were not found, or only very rarely, in stables, pigsties, and henpens, etc., which were frequented by animals. Such places are generally comparatively dry, constantly disturbed, and warmed by the presence of horses and cattle at night.

A number of the *Anopheles* collected by us have been kept in a damp cage in the animal house of the Thompson Yates laboratories, no food having been supplied; only two of the number have died during the month we have kept them in this condition. There can, therefore, be no doubt that mosquitoes of both genera 'hibernate' during the winter months in England, and it seems certain that not only the adults but, from Dr. WRIGHT's experiments, the larval forms also provide for the continuation of the species during the cold weather.

It is of interest to note that among the numbers of mosquitoes of both genera collected by us a male was never found; and, moreover, that all the females with

which we carried on a number of experiments or which we dissected had been fertilized (proved by the presence of spermatozoa in the spermatheca, or by the hatching out of larvae from deposited eggs).

A number of experiments relating to the bionomics of the English *Anopheles maculipennis* are at present being undertaken, and we hope to be able to communicate the detailed results at a later date. Some interesting facts may, however, be recorded. If these mosquitoes be kept in a *dry* cage they die in a few days—whereas, as stated above, they can be kept probably for months in a *damp* cage in the cold, during which time they preserve, what we propose to call, the ‘hibernating’ attitude. On introducing them into a warm room they quickly become active, and both *Anopheles* and some species of *Culex* eagerly feed on blood on inserting the hand into the cage, darkened by covering with a cloth. They then feed eagerly every day for four or five days, but subsequently only occasionally. Eggs were laid on the fifth, sixth, seventh, and eighth days, which hatched out in twenty-four or forty-eight hours. It was noted that many of the *Anopheles* died after depositing a batch of eggs.

We have further observed that having once fed on blood, it is necessary to continue the feedings at least every other day, otherwise the ovaries cease to develop and the insects die, though water is supplied. This confirms our experiments made in West Africa,* where by regular feeding we were able to keep *Anopheles costalis* and *funestus* alive for a considerable period; while in the present case of *A. maculipennis* which had been hibernating, most of them died soon after laying eggs.

On the 19th April* of this year, during a period of about two weeks of fine warm weather, we made another visit to the farms at which we had on previous occasions never failed to collect *Anopheles*. At one farm, three or four *Anopheles maculipennis* were seen, but being so very active only two were caught. At another some fifty were seen, but only ten caught, and these with great difficulty. The mosquitoes were exceedingly active, flying immediately the light of the candle fell upon them, and directly the bottle was placed near them. This activity strikingly contrasted with their slow, lazy movements of the previous week during colder weather. Many hundreds of *Culex* of different species were seen, their increased activity was also noticeable.

On the 23rd of the same month* another visit was made to these farms, but no specimen of *Anopheles* was seen in the sites where previously so many had been captured. At the same time other farms up to now not visited were examined without success. At one farm, however, we were allowed to search the whole house, and here found five specimens of *Anopheles maculipennis*, gorged with blood and showing developing ovaries, in the attics in which several Irish farm labourers slept,

* Report of the Liverpool Malaria Expedition to the Nigeria, 1901, p. 37-45

on the ceiling of the staircase, and on the ceiling and walls of the kitchen. In the other less-used and more cleanly-kept rooms none could be found. The visit was made about mid-day.

On the 5th of May,* at a farm near Chester, one female and two males, *Anopheles bifurcatus*, were captured in the laundry in the dusk of the evening. An examination of other parts of the farm was unsuccessful, except in the cellar, where the crowd of *Culex* was found to have left the wall, and had collected near the only possible exit (a fine wire grating of perforated zinc in the wall) where myriads had been killed in their endeavours to escape.

The *Anopheles* caught on the 23rd of April* we firmly believe to have hibernated during the winter months, and at that time were developing ova for the first time this year, having frequently during the winter and early spring months examined many farms in this district.

* The figures below give the mean daily temperature for each week throughout March, April, and May, in this district.

For week ending March	7	-	-	-	-	42.3° F.
"	"	"	14	-	-	43.1° F.
"	"	"	21	-	-	38.4° F.
"	"	"	28	-	-	35.8° F.
"	"	April	4	-	-	41.5° F.
"	"	"	11	-	-	45.2° F.
"	"	"	18	-	-	44.2° F.
"	"	"	25	-	-	57.0° F.
"	"	May	2	-	-	49.5° F.
"	"	"	9	-	-	49.9° F.
"	"	"	16	-	-	52.1° F.
"	"	"	23	-	-	54.8° F.
"	"	"	31 (8 days)	-	-	58.4° F.

THE FLORA OF THE CONJUNCTIVA
IN HEALTH AND DISEASE

THE FLORA OF THE CONJUNCTIVA IN HEALTH AND DISEASE *

By A. STANLEY GRIFFITH, M.D. VICT.

ALEXANDER FELLOW IN PATHOLOGY

INTRODUCTION

The large and varied supply of ophthalmic material available in the eye clinic of the Royal Infirmary and in the Parish Infirmary of Liverpool, has afforded me the opportunity for the work embodied in my thesis.

I should like at the outset to express my thanks, in the first place, to Dr. ALEXANDER, the visiting surgeon of the Parish Infirmary, from whose wards I have obtained the bulk of my material, and also to Mr. BICKERTON, Ophthalmic Surgeon to the Royal Infirmary.

The research has been conducted with the following objects in view :—

- (a) To determine the flora of the normal conjunctival sac.
- (b) To compare the pathogenic properties of organisms occurring in healthy eyes with similar organisms found in diseased eyes.
- (c) To investigate the causal agents of the various suppurative inflammations of the conjunctiva met with.

The first part is a record of the results obtained in the bacteriological investigation of a number of healthy and diseased conjunctival sacs with a tabulated series of experiments on rabbits' eyes with some of the principal organisms isolated.

Further, to determine whether pyogenic cocci could be found in the normal conjunctival sac and in how far they possessed virulent properties as compared with similar organisms found in diseased eyes.

Apart from its scientific interest such an investigation is of the greatest importance to the ophthalmic surgeon not only in operative procedures but also in elucidating obscure points in the etiology of conjunctival disease.

In each case examined all the organisms cultivated have been noted and, for those that could not be named, a brief account of the principal cultural features has been given.

Special prominence has been given to the numerous varieties of bacilli resembling the diphtheria bacillus which occur in the conjunctival sac, and which correspond in a marked way to the different forms of diphtheria bacillus isolated by recent observers from the throat and nasal cavities.

* Presented in the form of a Dissertation to the Victoria University for the Degree of M.D. 1901.

SYNOPSIS OF CONTENTS

PART I—THE FLORA OF THE NORMAL AND DISEASED CONJUNCTIVAL SAC

1. Source of Material.
2. Methods.
3. Description of organisms found in the normal conjunctival sac.
4. Summary of all the organisms found in the diseased conjunctival sac.
5. Experiments to determine the rate of disappearance of organisms from the normal and diseased conjunctivae.
6. Experiments to determine the pathogenic properties of the organisms found in the normal conjunctival sac.
7. Experiments to determine the pathogenic properties of the organisms found in the diseased conjunctival sac.
8. Conclusions.
9. Previous work on the subject.

PART II—THE ORGANISMS ASSOCIATED WITH SUPPURATIVE INFLAMMATIONS OF THE CONJUNCTIVA

1. Methods.
2. Conditions examined—
 - (a) Ophthalmia caused by the gonococcus.
 - (b) Ophthalmia caused by the Koch-Weeks bacillus—
 - Description of cases.
 - Biological characters of Koch-Weeks bacillus.
 - Inoculation experiments.
 - (c) Muco-purulent catarrh caused by the Koch-Weeks bacillus—
 - Description of cases.
 - Previous work on the Koch-Weeks bacillus.
 - (d) Ophthalmia associated with *B. Diphtheriae* and *Streptococcus pyogenes*.
 - (e) Ophthalmia caused by *Streptococci*.
 - (f) Ophthalmia caused by *Staphylococci*.
 - (g) Ophthalmia caused by *Staphylococcus albus*.
 - (h) Granular ophthalmia—
 - Muco-purulent catarrh associated with small granules.
 - Large granules (true trachoma).
 - Chronic trachoma with cicatricial thickening.
 - Histology of the trachoma granule.
 - Literature.
 - (i) Diplobacillary conjunctivitis.
 - (j) Diseases of the lachrymal sac—
 - Mucocele.
 - Acute dacryocystitis.
3. Unclassified cases.

PART I

THE NORMAL FLORA

The results given in this section are based upon an examination of 210 cases, in which the conjunctiva was apparently healthy. The material for this purpose has been collected chiefly in the out-patient department of the Liverpool Royal Infirmary from persons suffering from some refractive error or other disease not affecting the conjunctival mucous membrane. A few of the cases were taken from amongst my colleagues in the laboratory, and from the wards of the Liverpool Parish Infirmary, these latter being mainly children.

In collecting this series an endeavour has been made to include individuals differing as much as possible in age, sex, social position, and environment.

Whilst it is obvious that the number and character of the organisms temporarily inhabiting the conjunctival sac will depend largely upon the number of bacteria in the immediate neighbourhood of the individual, a more potent cause of contamination will be found in neglect of personal cleanliness. Dirty fingers, face, etc., are more likely to infect the conjunctiva with pyogenic cocci than the atmosphere, and these cocci will in all probability possess more virulent properties. It might be expected that the number of pyogenic cocci would vary in inverse ratio to the cleanliness of the individual and his surroundings. My cases have shown this expectation to be well founded.

Great care has been exercised in the selection of the cases. All those giving a history of 'gumming' of the lids in the morning, or sensation of grit in the eyes, etc., being discarded, as well as those showing abnormal redness or other apparent sign of disease.

*The method adopted in each case was as follows :—*The lower lid was everted, and the conjunctiva gently stroked with the loop of a sterilized platinum wire, until the loop had become charged with lachrymal fluid. A serum tube was inoculated by smearing the fluid evenly over the surface, and incubated at 38°C.

It was occasionally very difficult to obtain even a loopful of lachrymal fluid, but slight mechanical stimulation generally sufficed to produce a free flow of tears. The platinum loop was used principally on account of its convenience, and because it was thought that the organisms in the sample would be sufficiently representative of the total bacteriology of the sac.

In some of the cases a sterile cotton-wool swab was used. With this the whole of the lower conjunctival fornix was swabbed out, and after being rubbed well over the surface of serum the swab was placed in broth.

Although this method may remove from the sac a greater number and variety of organisms, it is open to some objection in that in the operation of smearing over

the surface of the medium many organisms will remain entangled in the wool, and in the subsequent broth culture they will either not grow or be overgrown by other organisms to which broth is a more suitable medium.

It has been noted in many cases that a far larger number of colonies, particularly of the xerosis bacillus, has been produced by the use of the loop than by the use of the swab.

Solidified horse-serum was used throughout for the primary inoculations ; in previous investigations it was observed that some of the organisms, notably the xerosis bacillus, present in the sac grew with great difficulty in primary culture on agar-agar, gelatine, etc.

After inoculation the tubes were generally kept in the incubator for forty-eight hours before examination ; at the end of twenty-four hours it was often impossible to detect any growth with the naked eye, whilst at forty-eight hours an abundant growth was revealed. Even those organisms which from other situations presented a good growth at twenty-four hours here produced a very inconsiderable growth.

The loopful of fluid was smeared well over the surface of the serum and the resulting colonies were so perfectly discrete that pure cultures of the different organisms could easily be obtained.

The following table shows the source of the material and the percentage of sterile sacs in the different groups of individuals.

TABLE I

No.	Source	No. of Sacs examined	Sterile Tubes	Method	Percentage Sterile
1	Workers in Laboratory	12	8	Platinum loop	66·6
2	Liverpool Parish Infirmary (Children)	40	11	Platinum loop and Diphtheria swab	27·5
3	Liverpool Royal Infirmary (a) Children and Adults	146	25	Platinum loop	17·1
	(b) Adults	12	3	Diphtheria swab	25·0
	TOTAL	210	47		

TABLE II

ORGANISMS FOUND IN THE NORMAL CONJUNCTIVAL SAC

Xerosis bacillus (Table IV, No. 1)	120 times
Staphylococcus epidermidis albus (Welch)	47 "
Staphylococcus pyogenes aureus	8 "
Staphylococcus pyogenes citreus	1 "
Staphylococcus pyogenes albus	14 "
Staphylococcus cereus flavus	1 "
Staphylococcus cereus albus	2 "
Streptococcus pyogenes longus	8 "
Streptococcus brevis...	12 "
Pneumococcus (Fraenkel)	2 "
Bacillus lacunatus (Eyre)	9 "
Bacillus mesentericus ruber	2 "
Bacillus subtilis	1 "
Bacillus capsulatus mucosus	1 "
Bacillus coli communis	1 "
Bacillus striatus flavus	2 "
'Red bacillus'	2 "
Sarcina lutea	3 "
Micrococcus tetragenus	1 "
Proteus vulgaris	1 "
Penicillium glaucum	1 "
Cladothrix (white)	1 "
Cladothrix (brown)	1 "
Table III, No. 1	3 "
Table III, No. 2	1 "
Table III, No. 4	1 "
Table III, No. 5	1 "
Table III, No. 6	2 "
Table IV, No. 2	1 "
Table IV, No. 3	2 "

Only forty-seven out of the total number of sacs examined were sterile.

The xerosis bacillus and bacilli of the diphtheria group were found one hundred and twenty-three times, fifty-two times in pure culture, twenty-four associated with the staphylococcus epidermidis albus and forty-seven in mixed culture.

The frequency with which this bacillus occurred suggested that with a more complete examination many of the sterile sacs would have shown the presence of one or two organisms.

It was impossible to make such a systematic examination of all the sterile eyes. In a few cases, however, a number of tubes were inoculated from eyes which had been sterile to one examination, and usually in one of the tubes a single colony of xerosis bacillus or staphylococcus epidermidis albus was found. It is not unlikely

that at the time of examination some conjunctival sacs are absolutely free from organisms, but the frequency of the occurrence of the xerosis bacillus and its presence, or the presence of other organisms, after repeated examination makes the conclusion that the normal conjunctival sac is sterile only in a very small number of cases apparently inevitable.

The list of organisms show that the pathogenic bacteria do not occur very frequently, and the experiments with them on animals demonstrate that they have to a certain extent lost their virulence.

It seems, therefore, that although a conjunctiva is but rarely sterile, the organisms it usually contains would have little effect in prejudicing the result of an aseptic operation.

Certain operations on the eye are performed in the upper quadrants, and, as EYRE⁺ pointed out, the upper fornix conjunctivae seldom contains organisms, the frequent sterility of this part of the conjunctiva and the infrequent occurrence of pathogenic organisms explains why eye operations so rarely become septic.

Staphylococcus aureus was isolated eight times—in six instances from children, and only two from adult sacs; the latter were found to be considerably less pathogenic than some of those isolated from children's eyes.

Streptococcus brevis was not found once in adults, the *streptococcus longus* only twice, and in one of the two was not pathogenic to mice.

The frequent occurrence of both varieties of *streptococcus* in children's eyes was very striking, and in order to confirm the results twelve additional cases were examined; in these *streptococcus brevis* occurred four times, *streptococcus pyogenes longus* twice.

To produce quickly a quantity of *streptococcus longus* the following method was adopted:—Over a twenty-four hours' sub-culture on slant agar, sterile broth was poured sufficient to cover all the colonies, and incubated at 38° C.; in one day each colony had grown out into the broth as a delicate villous-like prominence, with the position of the colony as a base; further incubation produced more growth, and if the tube was kept perfectly motionless fairly long threads grew out into the broth; from these growths very beautiful microscopical specimens of chains could be made. The slightest movement of the medium precipitated the mass to the bottom of the tube.

In two cases a few transparent colonies were noticed, which consisted of oval cocci, morphologically similar to the pneumococcus; they quickly died out, and it was not possible to study their life history.

Staphylococcus epidermidis albus (WELCH) was observed on forty-seven occasions. RANDOLPH isolated it in eighty-five out of one hundred cases examined.

The 'red bacillus' resembled in biological characters *B. latericeus*. On all media it formed a brilliant red growth; on gelatine it grew abundantly, but did not liquefy the medium.

	develops a brownish-yellow centre. The surface is dry and glazed.	Streak.—Growth occurs when planted thickly as a flat, raised, translucent, slowly-extending growth with a light-brownish centre. The surface is dry and slightly wrinkled or striated. The edges are crenate. No liquefaction.	develops a brownish-yellow centre. The surface is dry and glazed.	Streak.—Growth occurs when planted thickly as a flat, raised, translucent, slowly-extending growth with a light-brownish centre. The surface is dry and slightly wrinkled or striated. The edges are crenate. No liquefaction.	develops a brownish-yellow centre. The surface is dry and glazed.	develops a brownish-yellow centre. The surface is dry and glazed.
URINE	No ammoniacal decomposition.	Non-motile.	Non-motile.	Stains well with all the aniline dyes. Stains by Gram's method.	Stains well with all the aniline dyes. Stains by Gram's method.	Stains well with all the aniline dyes. Stains by Gram's method.
MOTILITY	Non-motile.	Non-motile.	Non-motile.	Non-motile.	Non-motile.	Non-motile.
STAINING REACTIONS	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.

TABLE III

BIOLOGICAL CHARACTERS OF SOME ORGANISMS THE NAMES OF WHICH WERE NOT DEFINITELY DETERMINED

	1	2	3	4	5	6
MORPHOLOGY	Large cocci of different sizes. Majority occur in pairs, the points of contact being flattened. The diameter of the cocci is about 1.5 μ . Some are found singly, others in tetrads. In old cultures the cocci stain badly, and many large swollen-out bodies are seen.	Cocci spherical, some are slightly elongated in transverse axis. They occur singly, in pairs, and short chains. When in pairs the sides of contact are flattened. They are about twice as large as staphylococcus aureus. Cultures resemble micrococcus aurantiacus.	Slender bacilli, 2 μ to 3 μ in length. Early formation of oval terminal spores, attached in many cases to the slender bacillus. This bacillus has a strong morphological resemblance to the bacillus alvei.	Straight rods of varying length, some growing out into very long threads. Early formation of spores; the spores are oval, and grow in the centre of the bacillus whilst at each pole is a small deeply-stained spot of protoplasm. Length of bacillus, 4 μ to 5 μ .	Small spherical cocci in masses, the size of staphylococcus aureus.	Small slender bacillus, running in swarms like a proteus.
SERUM	A raised, circular, conical, translucent, brown colony. Margins radially striated.	Raised, circular, orange-yellow, viscous growth.	Translucent, flat, slightly-raised, whitish film, spreading quickly and tending to cover the surface of the serum.	Colonies brownish, translucent.	Colonies are circular, opaque, and of a rich cream colour, they quickly extend in diameter, some becoming $\frac{1}{2}$ inch broad.	Translucent, dirty-white colony, from the periphery of which processes are given off.
AGAR	Pale, white, well-defined growth. Centre whiter and more opaque than the margins. Edges undulating. Surface becomes finely granular. Single colonies are rounded, circular, and translucent.	Opaque, orange-yellow growth, raised in the centre with a coarse granular surface.	Plate cultures.—No deep colonies. On the surface a slightly-raised, translucent, spreading film. Streak. As on serum the growth tends to cover the whole surface of the agar.	Single colonies transparent and circular. When confluent there is a translucent, white, slightly-raised growth spreading a little laterally in a series of short, rounded off-shoots, the edges of which are transparent and feathery.	Streak.—In one day an abundant, raised, white growth, quickly acquiring a cream-yellow colour, especially in the centre. The growth extends laterally. The edges are well defined and undulating.	Deep colonies, elliptical and yellowish. On the surface grey, translucent colonies seen in 24 hours; these increase in size and have a rounded centre and margins made up of ridges and cracks.
POTATO	No growth was obtained.	Growth opaque, yellow, raised in the centre and not tending to spread laterally. Surface of growth dry.	Along the line of inoculation a narrow, slightly-raised, translucent, lemon-coloured growth with no tendency to spread laterally.	Scanty growth, as a narrow, slightly-raised, yellowish line which with age becomes of an orange colour. Microscopical appearance.—The bacilli are much thicker than on agar.	Very abundant, raised, dry, opaque-white growth. Grows abundantly in the condensation water. In a short time the growth becomes slightly yellow.	Light-brownish, smooth, translucent growth, which quickly acquires a reddish-brown colour. Growth occurs in the condensation water. Microscopically there are many thread forms.
BROTH	White sandy deposit.	Slight general turbidity.	No appreciable growth.	No appreciable growth.	Finely granular turbidity and thick white deposit.	General turbidity, forming in a week a thick, white scum and an abundant white deposit. Indol reaction.
GLUCOSE BROTH		Disseminated growth and acid reaction. The organism grows anaerobically.			General turbidity with an abundant white deposit and acid reaction.	General turbidity. No acid or gas formation.
LITMUS MILK	No change in the medium.	Yellowish deposited growth. No change in the medium.	There is a considerable amount of deposited growth, but no coagulation of the casein. The litmus is coloured pink.	Acid reaction. No coagulation of casein.	After 3 to 4 days the litmus is turned pink. In a week or more the litmus is destroyed, and the milk coagulated as a thick jelly.	Fair amount of deposited growth, but no change in the medium.
GELATINE	Plate. Colonies are minute, circular, and translucent. By reflected light they have a light-brown, finely granular appearance. Stab.—No growth in the depth. On the surface is a circular, flat, grey expansion, which spreads slowly and develops a brownish-yellow centre. The surface is dry and glazed. Streak.—Growth occurs when planted thickly as a flat, raised, translucent, slowly-extending growth with a light-brownish centre. The surface is dry and slightly wrinkled or striated. The edges are crenate. No liquefaction.	Plate.—Colonies minute, circular, opaque and orange yellow. Medium not liquefied. Streak.—Raised orange-yellow growth. Stab.—Raised orange-yellow growth on the surface; in the depth a number of opaque, light-yellow colonies.	Streak.—Growth very slow, appearing as a limited, translucent line, not extending laterally. Stab.—No growth in the depth. On the surface a whitish, translucent, flat expansion.	Plate.—Colonies on the surface are minute (a little smaller than streptococcus colonies), circular, and opaque-white. Deep colonies are spherical and white. Streak.—Opaque, silvery-white, irregular growth, the outer margins of which are transparent and feathery. The edges are crenate. Stab.—In the depth a number of minute, opaque, white colonies. On the surface is a white growth, extending laterally in a number of short, fern-like off-shoots.	Plate.—Minute, opaque-white, regular colonies, which by transmitted light have a granular structure and a dark-brown nucleus. No liquefaction. The colonies in a short time become yellow. Streak.—Raised, opaque-white growth with abrupt, well-defined margins. In a week the growth has become a brilliant flesh-colour. Stab.—Opaque, cream or flesh-coloured expansion on the surface. In the depth thick granular line in which after a time large whitish colonies with a brown nucleus develop.	Plate.—Minute, opaque-white granular colonies. Streak.—Raised, grey, silvery-white growth, spreading a little laterally. Margins raised and well-defined. Streak.—White semi-translucent growth, spreading a little laterally with a tendency to heaping in the centre. Margins finely serrate. Later the centre of the growth assumes a wrinkled aspect. Glucose gelatine shake No gas formation.
URINE	No ammoniacal decomposition.					
MOTILITY	Non-motile.		Non-motile.	Non-motile.	Non-motile.	Motile.
STAINING REACTIONS	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.	Stains well with all the aniline dyes. Stains by Gram's method.	Does not stain by Gram's method.	Stains with all the aniline dyes. Does not retain the stain by Gram's method.	Stains with aniline dyes, and by Gram's method.	Stains with aniline dyes. Does not stain by Gram's method.

No. 3 in Table III was a slender spore bearing bacillus corresponding to the description of the bacillus of COLOMIATTI; morphologically it resembles *B. alvei*, which was found on one occasion by EYRE in the normal conjunctival sac. The bacillus occurred in muco-purulent catarrh, but for convenience of description I have included it in the description of organisms obtained from healthy eyes.

Many varieties of bacilli resembling in some particular the *B. diphtheriae* have been isolated from the conjunctiva, and forms varying from a short regular bacillus not forming typical involution forms and a form indistinguishable from the diphtheria bacillus in all its reactions have been obtained.

A table (IV) has been drawn up describing the cultural peculiarities of a few of those bacilli which have features sufficiently different to distinguish them from other members of the group. In this table, for convenience of description and comparison, has been included two varieties (4 and 5) only noticed in pathological conditions.

The organism known as the xerosis bacillus is the most common inhabitant of the conjunctival sac.

In two hundred and ten examinations it was found one hundred and twenty times, and it is not improbable that by using a larger amount of fluid it might have been found in a great many of the remaining cases.

In frequent instances so enormous must have been the number of organisms in the conjunctival sac, one loopful of fluid produced on the surface of the serum over two hundred colonies of this bacillus in pure culture. It was very common to produce from thirty to one hundred colonies in one inoculation.

To determine the time taken for colonies of xerosis bacillus to become visible to the naked eye, tubes were examined at different periods of incubation. On repeated occasions minute transparent colonies have been observed at the end of sixteen to eighteen hours, which have subsequently been proved to be the bacillus under consideration.

In morphological appearance the xerosis bacillus is very similar to the diphtheria bacillus, but differs from it in cultures. On serum the colonies are small, greyish-white, and very adherent to the surface of the medium; colonies of the diphtheria bacillus in the same time are much larger, whiter, and softer. On agar the xerosis colonies are very small, greyish, and translucent, and on media containing glucose growth is not accompanied by the formation of acid.

No. 2 (table IV) died out before a complete study had been made of its life history; it somewhat resembled a bacillus (diphtheroid I) isolated by EYRE¹ from milk.

No. 3 I have taken to be HOFFMAN's bacillus; it was identical in every respect with a HOFFMAN's bacillus derived from the throat.

¹ On the presence of members of the diphtheria group of bacilli other than the Klebs-Löffler bacillus in milk. *British Medical Journal*, August 18, 1900.

No. 4 only differed from the diphtheria bacillus in its slightly less granular appearance, and in its non-virulence. It is probably an attenuated diphtheria bacillus analagous to that occurring in a throat after an attack of diphtheria, or in the throats of apparently healthy children.

No. 5, although a short variety with few involution forms, produced abundant acid in glucose containing media ; it was, moreover, not pathogenic to guinea pigs.

In addition to the five varieties described in the table many intermediate forms have been isolated.

One form with highly segmented protoplasm formed colonies in serum and agar indistinguishable from those of the xerosis bacillus, but in litmus glucose broth at the end of five or six days acid was produced.

Another form, also similar to the xerosis bacillus in cultures, was in microscopical appearance a short oval bacillus closely resembling No. 5 ; it, however, did not produce acid in glucose containing media.

Other forms differing slightly either in morphology or in cultures from one or other member described in the table have been noticed, and it would appear possible to separate from the eye a complete series of bacilli beginning with the short regular form not producing acid in glucose and non-virulent, and ending with a typical diphtheria bacillus pathogenic to guinea-pigs.

LOUIS COBBETT¹ recognized five types of diphtheria bacilli occurring in the throat :—

1. Oval bacilli with an unstained septum. Young forms
2. Long, faintly stained, irregularly beaded bacilli
3. Regularly beaded bacilli. Streptococcal forms
4. Segmented bacilli
5. Uniformly stained bacilli

He found acid-producing bacilli identical in appearance both in culture and under the microscope with the diphtheria bacillus which were non-pathogenic.

Whilst the xerosis bacillus occurred one hundred and twenty times, other varieties of the diphtheria group were of comparative rarity in the normal conjunctival sac ; in diseased conditions, however, many varieties were often isolated from the same eye, HOFFMANN's bacillus occurring with almost as much frequency as the xerosis bacillus. It is worthy of note that the organism (No. 4) most nearly resembling *B. diphtheriae* was never found in the healthy sac, but was on several occasions found in the discharge from catarrhal ophthalmia and other non-diphtheritic inflammations.

1. *The result of 950 bacteriological examinations for diphtheria bacilli during an outbreak of diphtheria at Cambridge and Chesterton. Journal of Hygiene, vol. 1, No. 2.*

<p>MICROSCOPICAL APPEARANCES ON THE DIFFERENT MEDIA</p>	<p>On serum, in 48 hours, a few club-shaped organisms make their appearance, together with segmented bacilli, and metachromatic granules.</p> <p>On agar, in 24 hours there are many segmented forms, and a few club-shaped organisms are seen.</p> <p>On the seventh day the serum cultures do not take the methylene-blue stain, whilst the agar cultures stain well.</p> <p>(The fact that the serum cultures quickly lose their power of taking the stain was noticed also in the case of diphtheria bacillus which had many poorly-stained bacilli even on the third day.)</p> <p>In broth the bacilli occur in clumps; the individuals are shorter than those from serum.</p> <p>On the third day, clubbed and segmented bacilli are seen; also a few bipolarly-stained bacilli.</p>	<p>In 24 hours, on serum, there is a number of large segmented bacilli; a few of the bacilli are slightly clubbed.</p> <p>In seven days, involution forms very numerous; segmented forms abundant; there are clubs, segmented ovals, and a few bipolarly-stained bacilli.</p> <p>On agar, in 24 hours, majority of bacilli are long and segmented; there are a few clubs and segmented ovals.</p> <p>Metachromatism present.</p> <p>In broth, the bacilli occur in clumps, and early show segmented forms and clubs.</p>	<p>On serum, on the third day, a few short bacilli are seen with granular staining.</p> <p>On the ninth day, some of the bacilli are seen to have slightly enlarged ends, resembling miniature clubs.</p> <p>On agar, in six days, bipolar staining is seen.</p> <p>On potato, the bacilli are all short and stained at the poles, resembling diplococci.</p> <p>Metachromatism is seen.</p> <p>In broth, a few stain at the poles, but otherwise, after 10 days, there are no irregular forms.</p> <p>In broth, they occur in little clumps.</p> <p>On glycerine-agar, on the fifth day a few short segmented forms are seen.</p>	<p>Involution forms appear on the fourth day in serum cultures as large segmented bacilli; later, peg-top forms appear, and bacilli with polar staining.</p> <p>On agar, involution forms are seen in 24 hours, as segmented club-shaped bacilli.</p> <p>In three days the segmented bacilli have increased in number, and many perfect, club-shaped bodies are seen.</p> <p>Many of the bacilli have become swollen-out in the centre forming spinules.</p> <p>The spinule is a characteristic involution form of this bacillus, and is found in quantity in all cultures.</p> <p>In broth the bacilli occur in small clumps, the individuals being long and slender.</p> <p>In three days, segmented, spindle, and club-shaped bacilli are seen.</p> <p>On potato, in seven days, many and beautiful clubs are formed.</p> <p>On gelatine in seven days no clubs are seen, but many occur that are swollen-out in the centre.</p>	<p>The bacilli from the primary culture adhered so closely together that it was with difficulty that films were spread, but continual sub-culture on agar caused them to lose, to some extent, the property of clumping together in large masses.</p> <p>On the different media the microscopical appearances are fairly constant; the only irregular forms that appear are a few slightly longer bacilli with segmentary staining, having sometimes one end slightly swollen-out into a rounded knob.</p> <p>On potato, there is a very close resemblance to diplococci.</p> <p>After the third or fourth day the bacilli have lost their power of staining with methylene-blue.</p> <p>In broth, the bacilli are grouped together in masses of various sizes, which closely resemble collections of cocci.</p> <p>In addition to the short forms are seen slightly longer bacilli with three or four segments.</p> <p>Occasionally one of these longer forms occurred with one extremity slightly swollen.</p> <p>After sub-culture on agar for nearly six months the bacilli show a tendency to become longer and thicker in the centre with the formation of more pronounced involution forms.</p>
<p>STAINING REACTIONS</p>	<p>Stains with all the aniline dyes.</p> <p>Stains by Gram's method.</p>	<p>Stains by Gram's method.</p>	<p>Stains with all the aniline dyes.</p> <p>Stains by Gram's method.</p> <p>Cultures three or four days old stain badly with methylene-blue.</p>	<p>Stains with all the aniline dyes.</p> <p>Stains by Gram's method.</p> <p>Cultures on serum on the third day stain badly with methylene-blue, whilst cultures from agar, in marked contrast, stain well even after seven days.</p>	<p>Early cultures stain well with all the aniline dyes, but old cultures do not stain at all with methylene-blue, and only lightly with fuchsin and methyl violet; involution forms stain deeply.</p> <p>Stains by Gram's method.</p>
<p>MOTILITY</p>	<p>None.</p>	<p>None.</p>	<p>None.</p>	<p>None.</p>	<p>None.</p>
<p>CHROMOGENICITY</p>	<p>None.</p>	<p>A light-brownish colour is produced in the serum cultures.</p>	<p>In certain cultures a light-yellow colour develops.</p>	<p>On stab-agar a slight tinge of yellow appeared in the growth.</p>	<p>Cream-yellow colour, which does not infiltrate the medium.</p>
<p>GAS PRODUCTION</p>	<p>None.</p>	<p>None.</p>	<p>None.</p>	<p>None.</p>	<p>Strong acid reaction in less</p>

acid production in 24 hours

SUMMARY OF ORGANISMS FOUND IN THE DISEASED CONJUNCTIVAL SAC

The varieties of organisms isolated from inflamed eyes do not differ to any very great extent from the organisms isolated from healthy eyes. In an individual inflamed eye, however, it was very common to find four or five or even more varieties occurring together, whilst from a healthy eye it was very unusual to cultivate more than two or three different kinds of organisms.

After an inflammation of the eye had lasted for a little time, one or other of the pyogenic cocci was commonly found in the discharge, and, as a result of inoculation in a rabbit's eye and in a guinea-pig subcutaneously, it was found to possess considerably more virulence than a similar organism obtained from a healthy sac. There is very little doubt, therefore, that the ordinary pyogenic cocci occurring in chronic diseases of the conjunctiva contribute in some measure to the severity and continuance of the inflammation, and are probably in many cases, particularly in chronic conjunctivitis and in chronic trachoma, the only remaining cause of the continued inflammation.

In illustration of the fact that the pyogenic cocci on the inflamed conjunctiva possess an added virulence, I will mention a case of suppuration of the eye-ball following an operation for cataract. The patient had a little chronic catarrh of the conjunctiva with morning discharge, which was treated with antiseptics for some time before operation was considered advisable. The inflammation of the eye-ball which followed the operation shows how tenaciously suppurative organisms adhere to the hypertrophied conjunctiva, and how extremely difficult it is for antiseptics to act effectually on every corner of a roughened and pitted membrane.

From the purulent discharge in this case cultures of *B. xerosis* and *staphylococcus aureus* were obtained. The *staphylococcus* inoculated on the conjunctiva of a rabbit caused a very intense conjunctivitis.

A fact of aetiological significance is seen in the occurrence of certain organisms in the normal conjunctival sac, which, under suitable conditions, may give rise to an ophthalmia. *B. lacunatus* (Eyre), *streptococcus longus*, *staphylococcus aureus*, and *albus*, have been met with in the normal conjunctival sac and as causal agents of inflammatory processes. It is not improbable that the Koch-Weeks bacillus can reside in the healthy conjunctival sac without causing inflammation.

ORGANISMS FOUND IN THE DISEASED CONJUNCTIVAL SAC

Gonococcus

Koch-Weeks bacillus

Bacillus diphtheriae

Streptococcus pyogenes longus

Staphylococcus pyogenes aureus

Staphylococcus pyogenes albus
 Staphylococcus epidermidis albus
 Staphylococcus cereus albus
 Staphylococcus cereus flavus
 Staphylococcus citreus
 Staphylococcus brevis
 Pneumococcus (Fraenkel)
 Bacillus lacunatus (Eyre)
 Bacillus xerosis
 Bacillus subtilis
 Bacillus capsulatus mucosus
 Bacillus coli communis
 Bacillus enteriditis (Gärtner)
 Cladothrix (white)
 Penicillium glaucum
 Proteus vulgaris
 Sarcina lutea
 Sarcina aurantiaca
 Sarcina alba
 Bacillus of Colomiatti. Table III, No. 3
 Bacillus striatus flavus
 Table III, No. 1
 Table III, No. 6
 Table IV, No. 4
 Table IV, No. 5

COMPARATIVE STUDY OF THE RAPIDITY WITH WHICH ORGANISMS ARTIFICIALLY
INTRODUCED ARE REMOVED FROM THE HEALTHY AND DISEASED CONJUNCTIVAE

METHOD.—A pure culture of some easily detected organism was inoculated on the conjunctiva. A small quantity of lachrymal fluid was taken after the lapse of varying intervals of time by means of a sterile cotton-wool swab, which was smeared well over the surface of agar.

Experiment 1.—A loopful of sarcina lutea was introduced into a perfectly healthy rabbit's conjunctiva. The organism had entirely disappeared in eighteen hours.

Experiment 2.—Two loopfuls of bacillus coli were introduced into the healthy conjunctival sac of a rabbit. The disappearance was not so rapid as with the sarcina. In twenty-four hours eighteen colonies were grown, and in forty-eight hours, six; at sixty hours the organism was no longer present.

Experiment 3.—Two rabbits were taken whose conjunctivae were in a condition of chronic inflammation.

One loopful of bacillus coli was inoculated into each of the four eyes, and cultures were made on successive days. A growth of the bacillus was obtained up to the ninth day in two cases, and to the tenth and thirteenth in the remaining cases.

In one case, for the first four days, the organism occurred in gradually decreasing numbers, but on the fifth day there was a sudden increase; from this time there was again a decreasing number of colonies obtained, when on the tenth day there was a still further increase. On the thirteenth day and subsequently no colonies were obtained.

In another case there was no increase in the number of bacilli removed until the ninth day. In all the eyes the period during which the inoculated organism could be recovered was prolonged as compared with that observed in the case of the normal eyes.

These experiments show not only that organisms remain for a longer time in the diseased conjunctival sac than in the healthy, but also that organisms are capable of living and multiplying in the folds of the hypertrophied mucous membrane.

PATHOGENESIS

A number of experiments with the organisms isolated has been performed on animals.

It was not thought necessary to extend the experiments to every one of the common saprophytes of the air and to those bacteria which manifestly could have neither an effect in producing or continuing an inflammation.

Experiments were first performed on the conjunctiva of guinea-pigs, kittens, and rabbits, but the small size of guinea-pigs' conjunctival sacs and the unsuitability of kittens caused these animals to be rejected in favour of rabbits.

The rabbit possesses many advantages over other animals; the upper lid can with the greatest ease be everted and the whole conjunctival membrane exposed; the area of the membrane is considerable; slight friction with a platinum loop seems to cause the animal no inconvenience, and experiments and observations can be made with a minimum amount of movement of the subject.

In addition, the pathogenicity of certain organisms has been tested by inoculation in suitable form in guinea-pigs and mice.

The methods employed have been the same in every instance, and for purposes of comparison the amount of material has been the same in each case.

In the eye experiments the course pursued was as follows:—As early as possible after the primary inoculation the organism was subcultivated on agar or on

serum; a little of this subculture was taken on the loop of a platinum wire and smeared over the whole surface of the rabbit's conjunctiva with gentle friction. Where this rule is departed from mention will be made.

The experiments show that staphylococci from the normal conjunctival sac were considerably less virulent than similar staphylococci occurring in an inflamed conjunctiva. One loopful of a one day old agar culture of any of the staphylococci obtained from a healthy sac produced no reaction in a rabbit's conjunctiva when introduced without injury to the surface of the membrane, whereas simple introduction of staphylococcus aureus, and in one instance of staphylococcus albus, obtained from inflamed eyes was followed by a very appreciable inflammatory reaction.

With gentle friction, however, a very severe reaction followed inoculation of pyogenic staphylococci obtained from inflamed eyes, whilst of the staphylococci obtained from the healthy conjunctiva, staphylococcus aureus was the only one which produced any marked reaction, and this reaction was decidedly less than the inflammation produced by a staphylococcus aureus derived from an inflamed eye.

The following tables show at a glance the methods employed, and the results obtained, in each experiment.

TABLE V. INOCULATION EXPERIMENTS ON THE CONJUNCTIVA. NORMAL FLORA

No.	Organism	Age of Culture	Medium	Amount	With or without friction	Animal	Result
1	Xerosis bacillus	1 day	Serum culture	6 loops	+	Rabbit	No reaction.
2	St. epidermidis albus	1 day	Agar culture	1 loop	+	do.	At the end of 24 hours there was slight reddening of the conjunctiva without discharge. The appearance was normal at the end of 48 hours.
3	St. pyogenes albus	1 day	Agar culture	1 loop	+	do.	1st day.—There was a little white pus in the internal canthus and conjunctival sac, and slight congestion of the palpebral conjunctiva. The vessels of the ocular conjunctiva were slightly dilated. 2nd day.—The inflammation was subsiding, but there was still a little discharge. 4th day.—The appearance was normal. In one case there was a little undue redness which remained till the fifth day.
4	St. pyogenes aureus (a)	1 day	Agar culture	1 loop	+	do.	At the end of the first day there was a slight discharge with redness of the palpebral conjunctiva and slight dilatation of the vessels of the ocular conjunctiva. The membrane was practically normal before the end of four days.
5	(b)	1 day	Agar culture	1 loop	+	do.	In 24 hours there was fairly severe reaction, muco-purulent discharge, and redness of the nictitating membrane. The vessels around the cornea and the conjunctival vessels of the upper and lower lid were engorged. There was photophobia. The inflammation decreased after 36 hours and the discharge became less, ceasing at the end of four days. The redness of the palpebral conjunctiva remained for two days longer.
6	St. cereus flavus	1 day	Agar culture	1 loop	+	do.	There was a little discharge in 24 hours, ceasing before 48 hours, and moderate congestion lasting three days.
7	Streptococcus pyogenes longus	1 day	Agar culture	Whole of culture	+	do.	No reaction.
8	Bac. Lacunatus (Eyre)	1 day	Serum culture	Several loopfuls	+	do.	No reaction.
9	Bac. Mucosus	1 day	Agar culture	3 loopfuls	+	do.	No reaction.
10	The same, after passage through guinea-pig	1 day	Agar culture	3 loopfuls	+	do.	No reaction.
11	Bacillus coli communis	1 day	Agar culture	3 loopfuls	+	do.	No reaction.

TABLE VI
 INOCULATION EXPERIMENTS ON THE CORNEA
 NORMAL FLORA

No.	Organism	Age of culture	Medium	Method	Animal	Result
1	B. xerosis	1 day	Serum	The cornea was abraded with a sterilized needle loaded with a little pure culture	Rabbit	No opacity.
2	St. epidermidis albus	1 day	Agar	do.	do.	No opacity.
3	St. pyogenes aureus	1 day	Agar	do.	do.	Slight cloudiness around the point of inoculation in one day, which extended and increased in density up to the third day, when it began to diminish. On the second day vessels were seen growing out over the cornea from the ocular conjunctiva; these increased in size up to the third day, and formed a beautiful network over the opaque area. At the same time there was some conjunctivitis and discharge, but this cleared up in three days. In a week the opacity had disappeared and the vessels were obliterated.

TABLE VII. SUBCUTANEOUS INOCULATION EXPERIMENTS. NORMAL FLORA

No.	Organism	Age of culture	Medium	Amount	Animal	Weight	Result
1	<i>B. xerosis</i>	1 day	Serum	Growth on six tubes emulsified	Guinea-pig	520 grms.	No reaction.
2	<i>St. epidermidis albus</i>	1 day	Broth	3 c.c.	Do.		No abscess formation.
3	<i>St. cereus albus</i>	1 day	Broth	3 c.c.	Do.	495 grms.	No abscess formation.
4	<i>St. pyogenes albus</i>	1 day	Broth	3 c.c.	Do.	515 grms.	No abscess formation.
5	<i>St. pyogenes citreus</i>	1 day	Broth	3 c.c.	Do.	422 grms.	In 3 days a small lump was seen which was found to contain a little reddish pus. The organism was cultivated from the pus. Animal recovered.
6	<i>St. pyogenes aureus</i>	1 day	Broth	3 c.c.	Do.	500 grms.	In 1 day oedematous swelling; pus formed on the third day. On the fifth day death with metastatic abscesses. <i>Staphylococcus aureus</i> recovered from the pus.
7	<i>St. pyogenes aureus</i>	1 day	Broth	3 c.c.	Do.	700 grms.	Small indurated lump, which in 4 days did not form into an abscess. On the fourth day the animal was killed; there was no pus found at the point of inoculation.
8	<i>Streptococcus brevis</i>	1 day	Broth	1 c.c.	Mouse		Animal lived.
9	<i>Streptococcus pyogenes longus</i>	1 day	Broth	1 c.c.	Mouse	18 grms.	No reaction.
10	<i>Bacillus mucosus capsulatus</i>	1 day	Agar	Whole of agar culture emulsified	Guinea-pig		At the end of 5 days there was a large, indurated, painful swelling; animal looked ill and had lost weight. In 11 days the swelling had diminished. Animal suffered from diarrhoea. At the end of 15 days the animal was killed. At the point of inoculation there was a tumour the size of a small marble containing a thick white pus. Cultures = pure culture of <i>Bacillus mucosus</i> .
11	<i>Bacillus coli communis</i> inoculated into peritoneum	18 hrs.	Broth	3 c.c.	Guinea-pig		Death in 36 hours with general infection. P.-M. Increased amount of fluid in peritoneum; flakes of lymph in the fluid and on the intestines; congestion of mesenteric vessels. The lymph contained bacilli, some of which were in the cells. The blood contained the bacilli. Pure cultures were obtained from the peritoneal fluid and the blood, colonies in the latter case not being numerous.

TABLE VIII. INOCULATION EXPERIMENTS ON THE CONJUNCTIVA WITH ORGANISMS FROM INFLAMED EYES

No.	Organism	Source of organism	Age of culture	Medium	Amount	With or without friction	Animal	Result
1	Xerosis bacillus	Trachoma	1 day	Serum	6 loops	—	Rabbit	No reaction.
2	Staphylococcus epidermidis albus	Do.	1 day	Agar	1 loop	+	Do.	There was a slight reddening of the conjunctiva in 24 hours. The appearance was normal at the end of 48 hours.
3	Staphylococcus pyogenes albus	Dog Ophthalmia	1 day	Agar	1 loop	+	Do.	At the end of 24 hours the eyelids were 'gummed' together; there was great swelling of the upper lid and profuse discharge of white pus: the upper and lower conjunctival membrane was congested and swollen; the vessels of the nictitating membrane were congested, and there was a ring of dilated vessels around the cornea. The cornea was faintly milky all over, but principally in the upper quadrants. On the second day the oedema was a little less, the discharge remaining about the same in amount. The cornea was distinctly more opaque in the upper quadrants, and a few vessels were seen invading the corneal tissue. On the third day the inflammation commenced to diminish in intensity, and on the seventh day the eye was practically normal. The corneal opacity was the last to disappear.
4	Staphylococcus pyogenes albus	Corneal Ulcer	1 day	Agar	1 loop	+	Do.	In one day there was a fair amount of pus in the conjunctival sac and the internal canthus; some dried discharge around the roots of the eyelashes; congestion of the palpebral conjunctiva, and slight dilatation of the vessels of the ocular conjunctiva. The cornea was clear. The condition remained the same up to the second day, when the inflammation began to diminish, clearing up before the end of the fifth day.
5	Staphylococcus pyogenes aureus	Chronic Trachoma	1 day	Agar	1 loop	+	Do.	In 24 hours there was a large amount of yellowish-white discharge 'gumming' the lids together; intense injection of conjunctiva and nictitating membrane; dilatation of a few of the ocular conjunctival vessels. The upper eyelid was swollen and thickened; the cornea remained clear. On the second day the condition remained practically the same, but there was intense injection of the conjunctiva. Subsequently there was slow improvement, but the conjunctiva never seemed to quite recover its normal condition, the vessels remaining slightly enlarged and tortuous, the conjunctiva thickened and rugose.
6	Staphylococcus pyogenes citreus	Gonorrhoeal Ophthalmia	1 day	Agar	1 loop	+	Do.	At the end of 24 hours there was slight congestion, with a little white secretion in the internal canthus. The conjunctiva was normal in three days.
7	Streptococcus pyogenes longus	Chronic Trachoma	1 day	Agar	Whole of Culture	+	Do.	Beyond a little congestion, there was no reaction.
8	B. lacunatus (Eyre)	Trachoma	1 day	Serum	6 loopfuls	+	Do.	No reaction.

TABLE IX. INOCULATION EXPERIMENTS ON THE CORNEA

No.	Organism	Source	Age of Culture	Medium	Method	Animal	Result
1	Staphylococcus pyogenes albus	Dog Ophthalmia	1 day	Agar	The margins of the cornea at the junction of the two upper quadrants was abraded with a needle loaded with a little pure culture	Rabbit	<p>In addition to the corneal changes there was also a very severe conjunctivitis, with profuse discharge and oedema of the upper and lower lid. At the point of inoculation in one day there was a little point of pus; the cornea in the immediate neighbourhood was quite opaque, whilst the rest of the cornea was cloudy.</p> <p>The opacity increased until on the third day the whole cornea was opaque and vessels had grown for a short distance into the corneal substance. The margins of both upper and lower eyelids presented great irregularity of contour.</p> <p>On the fifth day the rabbit died. P.-M. The cornea was opaque especially in its upper half, vessels having grown for some distance into the corneal substance. There was pus in the anterior chamber, and the lens was swollen and opaque. On the top of the rabbit's skull, beneath the skin, was a small abscess cavity, the pus of which contained Staphylococcus albus, a few colonies of Staphylococcus aureus, and Streptococcus longus.</p>
2	St. pyogenes aureus	Acute Ophthalmia (Koch-Weeks bacillus)	1 day	Agar	do.	Rabbit	<p>In one day, at the point of inoculation, there was a small spot of pus with milkiness of the cornea in the immediate neighbourhood; the milkiness increased in extent and density up to the second day, extending from the point of inoculation to the median line of the cornea. The vessels of the sclerotic above the abrasion were greatly dilated and tortuous, and seemed to send branches into the corneal substance as well as the ocular conjunctival vessels.</p> <p>As a consequence of the presence of the Staphylococcus in the conjunctival sac there was, in addition to the above manifestations, an acute conjunctivitis.</p> <p>At the end of the second day the condition showed slight improvement, and on the fifth day the conjunctivitis had disappeared and the cornea showed no sign of opacity or vascularisation.</p>

TABLE X. SUBCUTANEOUS INOCULATION EXPERIMENTS

No.	Organism	Source	Age of Culture	Medium	Amount	Animal	Weight	Result
1	Xerosis bacillus	Membranous conjunctivitis	1 day	Serum	Growth on 3 tubes emulsified in 3 c.c. broth	Guinea-pig		No reaction.
2	Staphylococcus pyogenes albus	Dog ophthalmia	1 day	Broth	3 c.c.	Guinea-pig		Animal died in about 12 hours. Pure cultures obtained from the subcutaneous oedema fluid, but not from the blood or peritoneal fluid.
3	Staphylococcus pyogenes aureus	Muco-purulent Catarrh	1 day	Broth	3 c.c.	Guinea-pig	230 grms.	Animal died in about 24 hours. Pure cultures obtained from the oedema fluid and the peritoneal fluid; a few colonies were cultivated from the blood.
4	ditto	Catarrhal conjunctivitis	1 day	Broth	3 c.c.	Guinea-pig	520 grms.	In 3 days there was a large tense swelling in the region of the inoculation; the swelling increased in size, pointed and burst on the fifth day, leaving a raw surface the size of a shilling. The animal recovered.
5	Streptococcus pyogenes longus	Dacryocystitis	1 day	Broth	1 c.c.	Mouse	18 grms.	Death in 5 days from general infection.
6	Bacillus coli communis (inoculated into peritoneum)	Chronic conjunctivitis	1 day	Broth	3 c.c.	Guinea-pig		Animal died in 36 hours from general infection.

CONCLUSIONS

That the normal conjunctival sac contains organisms in a large proportion of cases.

That pyogenic organisms are only occasionally found in the normal sac, and, when they do occur, have to some extent lost their virulence.

That pyogenic organisms obtained from the inflamed conjunctiva are usually considerably more virulent than similar organisms obtained from the healthy conjunctival sac.

PREVIOUS WORK

Only within the last few years has any important work contributed to our knowledge of the bacteriology of the normal conjunctival sac. All investigations have been made to determine the frequency and pathogenicity of the pyogenic cocci and the percentage of sterile sacs.

MORAX¹¹ believed that the normal conjunctival sac was never sterile. He pointed out that pathogenic organisms are rarely found, and in a series of cases examined by him he never found a staphylococcus aureus or a streptococcus.

FICK⁵, in forty-nine observations, found the sac sterile six times, and in another series of fifty found the sac sterile thirty-six times. He isolated various bacilli which he had some difficulty in identifying; he also isolated staphylococcus aureus, micrococcus candidans, streptococcus and sarcina lutea.

GASPARRINI states that the micrococcus of pneumonia is found in a large proportion of healthy eyes. He injected fresh cultures into the anterior chamber or vitreous of rabbits producing panophthalmitis, and a plastic iritis and atrophy of the eye with older cultures.

FRAENKE⁶ found the healthy sac sterile in twenty-eight per cent. of his cases, the staphylococcus aureus or albus occurring ten times out of one hundred and fifteen examinations. He was unable to cultivate the xerosis bacillus, whilst FRAENKEL and UHTHOFF state that it is frequently present in normal eyes.

WIDMARK¹³ brought forward experimental evidence that the pus organisms when introduced into the conjunctival sac of rabbits did not produce catarrhal inflammation. On the other hand, when inoculated into the cornea an intense conjunctivitis resulted, together with keratitis and perforation of the cornea in fifteen per cent. of the cases.

LEBER and WEEKS obtained no result by inoculating staphylococcus aureus in the human conjunctiva.

GAYET found the staphylococcus aureus in the healthy conjunctival sac.

GOMBERT,⁷ with the bacteria isolated from the conjunctival sac, performed experiments on the rabbit's cornea; three were found to be pathogenic, producing opacity of the cornea; nine were non-pathogenic.

MAERTHEN¹⁰ described sixteen varieties of cocci, and two of bacilli ; the cocci included staphylococcus aureus and albus. Streptococcus was not found.

MACFARLAND says that the micro-organisms found in the normal sac are of common occurrence in the air. He encountered several bacilli not previously described (*Bac. hirsutus*, *Bac. coerule-faciens*, *Bac. circumscriptus*, *Bac. succinatus*, *Bac. violaceus flavus*).

LACHOWICZ⁸ examined sixty-three normal conjunctival sacs, of which sixty-nine per cent. were sterile. He concluded that the micro-organisms present came principally from the air, and that they only stayed there a very short time. In his experiments he showed that pure cultures of streptococcus and xerosis bacilli introduced into the conjunctival sac did not produce the slightest irritation. Staphylococci were also found.

GIFFORD, in his cases, found exclusively micrococci.

BACH frequently found the pus cocci in healthy eyes. He describes twenty-seven different micro-organisms, eighteen of which were micrococci. He says that in a large percentage of normal sacs bacteria may be demonstrated and that the conjunctiva must be regarded as constantly infected.

LAWSON⁹ in a series of two hundred cases found the healthy conjunctival sac sterile forty-one times. Serum was used throughout for the primary inoculations. In sixteen cases only were pyogenic cocci isolated ; staphylococcus pyogenes albus occurred six times ; staphylococcus aureus, once ; staphylococcus citreus, twice ; staphylococcus cereus albus, four times ; FRAENKEL's pneumococcus, twice.

Various non-pathogenic organisms occurred, of which staphylococcus epidermidis albus was found fourteen times. Inoculation experiments on the cornea of rabbits and guinea-pigs were without result. Staphylococcus aureus was not tried. He calls attention to the frequency of the so-called xerosis bacillus and the comparative infrequency of pyogenic organisms and their non-virulency. The xerosis bacillus was found in one hundred and eighteen tubes, ninety in pure culture.

RANDOLPH¹² made a series of experiments upon the conjunctivae of one hundred individuals. In thirteen cases the conjunctival sac was sterile, and out of the eighty-seven fertile tubes he observed that eighty-five contained staphylococcus epidermidis albus, whilst only two contained a bacillus. The value of these experiments is somewhat vitiated by the fact that in all the cases agar-agar was used as the culture medium. Xerosis bacillus, *Bac. lacunatus* (EYRE), and others will only grow readily on serum in primary culture. The experiments are of value in that, although a correct estimate of the bacteria of the conjunctival sac has not been arrived at, they show not only that the sac is rarely sterile but that the organisms generally found therein are of feeble pathogenic power.

Additional experiments were made to prove that the most effectual antiseptic is the conjunctiva itself, and that the use of germicides, such as corrosive sublimate, only handicaps the conjunctival mucous membrane in dealing with pyogenic organisms.

EYRE³ gives a detailed description of the microscopical and cultural features of the xerosis bacillus which he obtained from cases of follicular catarrh or trachoma. At the end of twenty-four hours on serum no growth was visible to the naked eye or microscopically, but after a period varying from thirty-six to forty-eight hours after inoculation an abundant growth made its appearance. In many of my inoculations growth was visible at the end of sixteen to eighteen hours.

EYRE⁴ examined a series of one hundred and fifty healthy sacs from seventy-six individuals, seventy-five of these were sterile. He isolated twenty-eight different varieties of organisms which included most of the pyogenic cocci; staphylococcus aureus occurred sixteen times; citreus, four; albus, thirteen; epidermidis albus, fourteen; cereus flavus, two; and streptococcus longus, three times.

Staphylococcus aureus was inoculated three times into guinea-pigs and was found to be pathogenic.

Staphylococcus citreus in one case possessed considerable virulence, whilst another had but feeble pyogenic powers. Staphylococcus albus was pathogenic to mice in two out of four experiments.

Streptococcus pyogenes longus in each of the three times inoculated in mice caused streptococcic infection, two mice dying in three days, the third in five days.

He pointed out that the alteration which is found to have taken place in the biological characters of organisms obtained from the conjunctival sac is evidence of a very real bactericidal action of the tears, and he further mentions that the rate of growth is appreciably decreased.

Experiments as to the length of time organisms remained in the conjunctival sac were made. A pure culture of *B. prodigiosus* was introduced into the healthy sac of a rabbit, and at varying intervals 0.05c.c. of lachrymal fluid was drawn off. The number of organisms rapidly diminished, and at three hours 0.05c.c. contained only three colonies, whilst at twenty-four hours no colony could be produced.

PART II

In dealing with the bacteriology of the suppurative inflammations of the conjunctiva I shall not give a complete clinical account of each disease, as most of them are too well-known to need any detailed description. But where necessary for the clear understanding of the exact disease under consideration, a few points in the clinical history will be noted.

A table of all the bacteria found in inflamed eyes has already been drawn up for comparison with those obtained from healthy eyes, and inoculation experiments have shown that the pyogenic cocci from inflamed eyes are, in a large proportion of cases, more virulent than the normal flora.

It is extremely probable that many of the chronic inflammations of the eye, such as cicatricial trachoma and chronic conjunctivitis, are due solely to the continued action of those cocci which have become lodged in the hypertrophied folds of the conjunctiva. In many cases, also, the pyogenic cocci may contribute in some measure to the severity and continuance of an acute inflammatory process.

The material was collected by means of a sterile cotton-wool swab and the platinum loop. In many cases the pus was taken from the upper fornix conjunctivae after the lid had been everted ; by this means a sample of pus was obtained free from any risk of contamination.

The chief medium was again horse-serum, but in many cases the discharge was divided equally over serum, serum-agar, agar-agar, and broth.

In all cases films of the discharge were made. When there was no discharge films were sometimes made of the lachrymal fluid ; the most careful examination of the film in the latter case often failed to show any organism with the exception occasionally of a bacillus presenting granular staining.

OPHTHALMIA CAUSED BY THE GONOCOCCUS

I. Eight cases of gonorrhoeal ophthalmia have been examined. With the exception of one child three years old they were all cases of ophthalmia neonatorum. The gonococcus was observed in large numbers in every instance.

The only point worth calling attention to in these cases was the absence of all other bacteria except the gonococcus in the early stages. Later, numerous and varied organisms could be cultivated. The conjunctiva, in the disorganized state resulting from the inflammation, appears to become a nidus for all bacteria which are deposited on it, and the hypertrophied folds of the mucous membrane provide an excellent soil for their growth and multiplication.

II. In these researches the Koch-Weeks bacillus has been found in four varieties of inflammation about the eye.

- (a) In acute ophthalmia.
- (b) In catarrhal ophthalmia.
- (c) In catarrhal ophthalmia in which the upper lid is covered with small miliary granules (follicular catarrh) and also in a few cases associated with large granules.
- (d) In mucocoele.

ACUTE OPHTHALMIA CAUSED BY THE KOCH-WEEKS BACILLUS

Although the Koch-Weeks bacillus is usually associated with muco-purulent catarrh, yet occasionally it produces a very acute inflammation which cannot clinically be distinguished from gonorrhoeal inflammation.

Four of such cases have come under observation ; two of the cases, before a microscopical examination was made, were diagnosed as gonorrhoeal ophthalmia ; the other two were suspected to be due to infection by the gonococcus.

The ages of the patients were seven months, twelve months, seven years, and thirty-four years, respectively. . The last named, a woman with a large family, had had a previous and similar attack a few months before (no history of discharge from the eyes in the family could be obtained).

In the babies, both eyes were almost simultaneously affected ; in the older patients one eye alone was inflamed at first, but after a week or ten days, when the inflamed eye had approached the normal, the disease appeared in the other eye.

Examination of the discharge, after films had been made in the usual way and stained with an aniline dye, showed enormous numbers of a short, slender bacillus.

In all four cases by far the greater number of the bacilli seen in the film was enclosed by leucocytes. Many were scattered about in the fibrin and between the cells, but those inside leucocytes far outnumbered those outside. A few of the leucocytes might be seen containing one or two bacilli only, but usually if leucocytes contained any at all, they were found absolutely packed with them.

It will be seen later that in less acute inflammations caused by the Koch-Weeks bacillus it was sometimes difficult to find a single leucocyte containing the bacillus.

This organism, sometimes, and more correctly, called the bacillus conjunctivitis, is a very slender, short rod closely resembling the bacillus of mouse septicaemia. Its length varies somewhat, but in discharge it rarely exceeds 1.5μ in length. It is very frequently seen with a constriction in the middle forming two distinct elements, each element having a slightly oval shape ; the dividing line is sometimes difficult to make out. The bacillus never forms chains.

CULTURAL PECULIARITIES

The cultivation of the bacillus conjunctivitis was very difficult. It does not grow on the ordinary laboratory media, and only occasionally on coagulated horse serum. Pure cultures, however, have been obtained on horse serum, serum agar, and human blood agar. On the two latter media a few colonies only grew on one occasion, and sub-cultures could not be obtained. On serum there was better success, and sub-cultures were made to the third and fourth generation. The saprophytic growth of the bacillus varies considerably, and seems to depend upon the period it has been exposed to the action of the lachrymal secretion. If cases are taken early, a good growth can be obtained on serum. In the four acute cases a good growth was obtained on serum, and the organism retained its vitality for some time. In mucopurulent catarrh of some duration a good growth was most difficult to obtain, and sub-cultures were not readily made.

During the course of these observations, REINHARD HOFFMANN,²³ in a paper on the Koch-Weeks bacillus, mentions that a medium composed of two parts of two per cent. glycerine-peptone agar and one part of human ascites fluid, mixed with sterile wether blood in the proportion of one to two, gave very good results, and that he was able to sub-cultivate the bacillus up to the twenty-fifth generation.

So far as my observations with this medium go, I have been unable to confirm HOFFMANN's statements.

On serum the colonies are minute, discrete, transparent, slightly raised growths, with a rounded conical centre and flat smooth margins. The colonies can only be seen with distinctness by the aid of a magnifying lens. In two days they have obtained their greatest magnitude. In one case they were found growing in association with a coccus, and in several cases they occurred in mixed culture with the xerosis bacillus.

Microscopically, the colonies consist of very slender, non-motile rods, varying in length but not in thickness; some of them grow out into fairly long curved threads. Many of the shorter bacilli are divided by a barely perceptible division. Most of the bacilli are cylindrical, but a few show slight irregularities of contour, which may be evidence of commencing subdivision. No chains or degeneration forms are seen.

They stain well with fuchsin, but lightly with methylene blue and dahlia; the stain is not retained by GRAM's method. They are non-motile.

Inoculation experiments on rabbits with pure cultures were unsuccessful, but with the mixed growth of Koch-Weeks bacillus and coccus a slight catarrh was produced, lasting for three days, and manifesting itself chiefly in the discharge of a small quantity of white muco-pus. Microscopically, the pus contained numbers of Koch-Weeks bacillus.

The interest of these cases lies in the fact that they may readily be mistaken for infection by the gonococcus. Clinically, in the case of the two children it was

impossible to differentiate between the two diseases ; they offered the clinical picture of a gonorrhoeal ophthalmia with the exception that the cornea did not seem to be endangered.

In the older patients the age, the character of the discharge, the previous attack, the absence of corneal complications and gonorrhoeal history would lead one to suspect that gonorrhoeal ophthalmia was not being dealt with. With regard to the character of the discharge, in the two latter cases the discharge, although profuse, was of a whitish colour and stringy consistence, and was with difficulty removed from the conjunctival sac ; gonorrhoeal pus is invariably yellow and friable.

The previous attack is a very important aid to diagnosis ; it lends colour to the supposition of REINHARD HOFFMANN²³ that the bacillus conjunctivitis is able to exist and lie dormant for a long time in the slightly hypertrophied folds of the conjunctiva resulting from a former attack, and become a means of propagation of the disease to other people and a danger to the patient in recurring attacks.

The woman must, in all probability, have been harbouring the bacillus in the recesses of her conjunctiva during the period between the two attacks, and, owing to lowered general vitality or diminished local resistance, the bacillus was able to again manifest its presence. In her first attack the disease remained confined to one eye, but in the second the other eye became involved ten days afterwards.

This form of ophthalmia differs from the classical muco-purulent catarrh in the severity of the symptoms ; it is characterized by intense pain, photophobia, and redness of the conjunctiva, profuse discharge and often large sub-conjunctival ecchymoses.

In the case of the woman, after the inflammation had subsided, the upper lid was everted, and the conjunctiva examined. The membrane was very congested, and presented a roughened appearance ; the roughness did not amount to actual miliary granulations.

III. MUCO-PURULENT CATARRH CAUSED BY THE KOCH-WEEKS BACILLUS

The Koch-Weeks bacillus varies considerably in virulence. In the disease now under consideration, the invasion of the conjunctiva by the organism causes only a mild type of inflammation, and, in comparison with the acute ophthalmia described above, frequently causes only slight congestion of the palpebral conjunctiva.

The discharge contains a large proportion of fibrin and occasionally forms a pseudo-membrane.

Microscopical films of the discharge show a large amount of fibrin enclosing polynuclear leucocytes, epithelial cells, and bacteria.

In recent cases the slender bacilli are very numerous, and if the inflammation be fairly acute many leucocytes are seen containing bacilli. The bacilli were often seen adhering to epithelial cells.

As the disease diminishes in severity the number of leucocytes containing bacilli, and the number of bacilli in the discharge, correspondingly diminish.

At first no other organisms could be observed in microscopical preparations, and in cultures from very early cases, besides the Koch-Weeks bacillus and a few colonies of the xerosis bacillus, no other organisms could be cultivated.

As the disease became more advanced the adventitious organisms increased in number and variety; the pyogenic cocci, and especially the staphylococcus aureus, became frequent inhabitants of the sac.

As convalescence approached the enormous increase in the numbers of the xerosis bacillus was very remarkable. In the small amount of discharge at this period the bacillus was sometimes so abundant that films appeared to be made from a pure culture.

PREVIOUS WORK ON THE SUBJECT OF INFECTION BY KOCH-WEEKS BACILLUS

The bacillus was first seen by KOCH²⁸ (1883) in the discharge from cases of acute catarrhal ophthalmia. He examined in Egypt fifty cases of ophthalmia in which he found two microbes; the one, NEISSER's gonococcus, associated with severe symptoms; the other, a small slender bacillus associated with mild symptoms. He ascribed the propagation of the disease to flies which were often seen covering the faces of children. Cultural experiments were unsuccessful.

In 1887 KARTULIS described the bacillus in Egyptian ophthalmia and corroborated KOCH's observations. He describes the bacillus in pure culture and succeeded in producing the disease in man in one out of six inoculation experiments. The description of his cultures would apply to the cultural appearances of the xerosis bacillus, but it is not unlikely that, since the slender bacillus may sometimes be found growing in association with or in the neighbourhood of other organisms, in his successful inoculation the culture contained Koch-Weeks bacillus.

In 1887 WEEKS⁴⁵ published a memoir in New York on the results of his investigations on catarrhal ophthalmia. He found in all his cases a small slender bacillus but was unable to obtain pure cultures, a club-shaped organism being always associated with it. Inoculation of the discharge on animals gave a negative result, but he was able to reproduce the disease in man by employing the mixed cultures.

In 1895 WEEKS⁴⁶ in a further communication stated that, in over a thousand cases of catarrhal ophthalmia examined by him, Koch-Weeks bacillus was a constant factor in producing the disease.

GROMAKOWSKI¹⁸ in eighteen cases of acute conjunctivitis found a slender bacillus which he concluded had an etiological relationship with an acute, highly epidemic conjunctivitis.

MORAX³¹ concluded that the bacillus was constantly present in muco-purulent catarrh. He described pure cultures of the bacillus and stated that inoculation of these cultures on his own conjunctiva produced acute inflammation.

He also further describes the histological appearances of a piece of his conjunctiva which was snipped off. The bacilli in sections of this tissue were with difficulty demonstrated in the superficial layers of the conjunctiva; in the deeper layers they could not be detected.

Later, JULER mentioned that he had met the organism in many, but not in all, cases of acute ophthalmia.

MORAX and BEACH³³ published a full account of their experiments with the bacillus which they cultivated on agar-agar with or without blood serum.

WILBRAND, SAENGER, and STAELIN⁴⁴ describe an epidemic of conjunctivitis in Hamburg in which two varieties of micro-organism were found, one of which was Koch-Weeks bacillus.

JULER, PANAS, and COPPEZ⁴ succeeded in observing the bacillus. COPPEZ draws attention to the often pseudo-membranous character of the inflammation to which I have already alluded.

GASPARRINI¹⁵ mentions the occurrence of the bacillus in Italy.

SIDNEY STEPHENSON^{40 & 41} gives a description of the outbreak and course of a small epidemic in the Central London District School, in which the characteristic small bacilli were found. He states that the presence of Koch-Weeks bacillus in the discharge is diagnostic of acute catarrhal ophthalmia, and is of the same opinion as CUENOD,⁵ that the club-shaped organism described by other writers on the subject is pseudo-diphtheritic.

He found the bacillus in three types of acute inflammation—

1. Classical catarrhal ophthalmia.
2. A form associated with large phlyctenulae in and about the conjunctiva.
3. A variety in which follicular enlargement is superadded, and also in a child suffering from chronic dacryocystitis.

He succeeded in growing the organism on serum-agar, but states that after three to four days it dies, showing degeneration forms.

WEICHSELBAUM and MULLER⁴⁷ (1898) published a very comprehensive work on the Koch-Weeks bacillus. They succeeded in isolating the bacillus in pure culture and establishing its etiological significance by successful inoculations on the human conjunctiva.

Within the last year Dr. REINHARD HOFFMANN²³ published an account of his cultural and inoculation experiments with this bacillus. He succeeded in growing it on swine serum-agar and 0.5 per cent. hydrocele fluid agar, but was able to sub-cultivate it only up to the fifth generation. He remarks that he obtained a growth on agar where a large amount of discharge was lying. The medium which gave him

the best results and enabled him to subcultivate the organism up to the twenty-fifth generation was a mixture of one part of the blood of a wether with two parts of a two per cent. glycerine-peptone agar and human ascites fluid in the proportion of one to two. Even in the oldest cultures he was not able to detect degeneration forms. Inoculation experiments on animals with the discharge and pure cultures were without result. Successful inoculation was carried out on himself and two of his colleagues, an inflammation resulting lasting over a week with the characteristic bacilli present. He concludes that Koch-Weeks bacillus is the cause of an acute, often croupous, very contagious eye inflammation in man, which may become chronic and result in a papillary hypertrophy of the conjunctiva.

Also that in the folds of the conjunctiva the bacillus can remain for a long time and be a possible source of transmission to other individuals and a danger to the individual himself in a re-awakening of the inflammation.

IV. ACUTE OPHTHALMIA IN WHICH *B. DIPHTHERIAE* AND *STREPTOCOCCUS PYOGENES* WERE ASSOCIATED

A case of acute ophthalmia was recently obtained which occurred in a female child one year old. One eye alone was affected. The inflammation was very intense, the discharge profuse, and the upper lid greatly thickened. Films of the discharge showed cocci, mainly in pairs, but also occurring in short chains; a few leucocytes contained cocci in chains of four and singly.

In one instance a leucocyte was seen containing a bacillus stained deeply at the poles. A few of these bacilli were also seen scattered about between the cells.

Cultures of *staphylococcus aureus*, *streptococcus pyogenes longus*, and a bacillus closely resembling *B. diphtheriae* were obtained.

A one day old serum culture of the bacillus was emulsified in one c.c. of broth and inoculated subcutaneously into a guinea-pig, 485 grammes in weight. The guinea-pig died in two and a half days. At the point of inoculation there was a greyish-white purulent exudate with a little surrounding oedema. Pure cultures of the bacillus were obtained from the pus at the point of inoculation.

V. ACUTE OPHTHALMIA IN A CAT CAUSED BY *STREPTOCOCCUS PYOGENES LONGUS*

The cat had, twenty-four hours before the observations were made, been bitten by a rat, the teeth of which had caused a penetrating wound of the right lower conjunctival membrane.

When seen, both eyelids were greatly swollen and oedematous; the edges of the lids adhered, and on separation a thick white pus exuded; the ocular and palpebral

conjunctivae were red and oedematous, large subconjunctival ecchymoses having taken place between the sclerotic and the ocular conjunctiva ; the cornea was bright and remained unaffected throughout. The acute symptoms quickly subsided without treatment, recovery taking place in four to five days.

Films of the discharge were made, streptococci in chains being the only organism observed.

VI. OPTHALMIA CAUSED BY STAPHYLOCOCCI

Two cases of inflammation of the eye have been examined in which microscopically and culturally only the staphylococci could be observed.

SYDNEY STEPHENSON⁴² records a case of acute ophthalmia, associated with pustular eruptions on the face and scalp, in which staphylococcus aureus and albus alone occurred. In my cases staphylococcus aureus and albus were found associated together. No other organism occurred to which the inflammatory condition could be ascribed.

The inoculation experiments with the organisms obtained from healthy eyes demonstrate that the staphylococci are able to cause an acute inflammation, and it is not improbable that in certain conditions of lowered general or local vitality the staphylococci which happen to be present in the conjunctival sac during such conditions are capable of originating an acute inflammation.

It is undoubted that in some chronic inflammatory conditions the continued inflammation is chiefly due to the presence of the organisms of suppuration.

VII. ACUTE OPTHALMIA IN A DOG CAUSED BY STAPHYLOCOCCUS ALBUS

The dog had had an attack of inflammation some weeks previously which had incompletely resolved.

This first attack followed directly on the formation of a discharging sore on the upper eyelid through the biting of flies ; the inflammation was not intense, and subsided in about three weeks, leaving the conjunctival mucous membrane slightly hypertrophied. After a month had elapsed the eye again became acutely inflamed. The eyelids were puffy and gummed together, enclosing a large amount of yellow pus. The conjunctiva was very red, swollen, and rough. One eye alone was affected at first.

In a short time after the onset of the inflammation the cornea was noticed to be becoming hazy ; the haziness was general and increased rapidly in density, until the whole cornea was densely opaque and pearly-white. The inflammation soon extended to the other eye, which exhibited the same symptoms in a modified degree.

After several weeks the inflammation became much less intense, and the opacity less, the cornea at last becoming perfectly clear and, apparently, in no way injured.

Examination of the discharge showed only cocci, many of which were in a state of sub-division. The cocci stained by GRAM's method.

Repeated cultural experiments were made, and in every case a number of opaque white colonies of a very virulent coccus was obtained.

On serum the colonies grew rapidly and caused a slight depression on the surface.

On agar an abundant opaque-white spreading growth was produced.

Milk was rapidly coagulated, the reaction becoming strongly acid.

Gelatine showed a large amount of liquefaction in twenty hours.

Culturally the organism resembled *staphylococcus pyogenes albus*, only producing equal results with greater rapidity.

The first attack was not investigated, but it is very improbable that the *staphylococcus* originated the disease.

The more likely assumption is that the disorganized conjunctiva was infected by the *staphylococcus* secondarily.

According to FLUGGE,⁹ *staphylococcus pyogenes albus* is more common than *aureus* among many of the lower animals.

VIII. GRANULAR OPHTHALMIA

The causal agent of trachoma has for a long time been involved in obscurity, and a series of cases has been examined to see if some light could be thrown on the subject.

SATTLER (1885) isolated a micrococcus from the trachomatous follicles in cases of Egyptian ophthalmia, and MICHEL (1886) who gave a more exact description of the coccus, made inoculation experiments which he believed established the etiological relationship to the form of ophthalmia with which it was associated. These researches have not been confirmed, and subsequent observers have not been able in many cases to find the micrococcus.

KARTULIS, FUCHS, and HOOR²² held the theory that trachoma is often of gonorrhoeal origin. DEMETRIADES⁶ considered Egyptian ophthalmia to be a combination of trachomatous disease with purulent ophthalmia as the gonococcus was always found in the discharge. In no instance in the series of cases described below was the trachomococcus cultivated, or the gonococcus observed in the discharge.

It was considered imperative, in order to find the exciting cause of trachoma, to obtain the cases in the early stage. This presented some difficulty, in that granules are generally of slow formation, and it was not possible to tell whether an inflammation

or a catarrh would eventually terminate in trachoma. But, owing to the opportunities of observing children with eye inflammations in the Workhouse Infirmary, it has been noticed that many children admitted with simple muco-purulent catarrh have returned at some period after their discharge suffering from granular lids.

In some instances, all the members of a large family have been inmates of the infirmary at the same time, some suffering from muco-purulent catarrh, others from granular lids.

Old cases of trachoma, although possessing some bacteriological interest, are useless for the purpose of ascertaining the primary cause of the condition, inasmuch as the organism initiating the disease has probably long since disappeared.

The bacteriology of granular conjunctivitis has been described in three sub-divisions :—

- (a) Early cases showing very small granules scattered over the whole surface of the upper conjunctiva.
- (b) Later cases with large granules.
- (c) Old chronic cases with no characteristic granules but a large amount of cicatricial contraction.

In the first series of cases there may or may not be discharge; the discharge is usually not abundant, and many most pronounced granular lids have only a little discharge 'gumming' the lids in the morning.

In a few instances the small granules, when treatment has been neglected, have been observed to increase in size and merge into the second group.

In addition to the large granules the upper lid often shows a certain amount of new formation of fibrous tissue, causing slight thickening of the lid and alteration in the contour of the palpebral fissure.

The last stage of the disease is marked by the total disappearance of the granules, distortion of the upper lid with dense fibrous tissue, contraction of the conjunctival sac, pannus and other corneal complications.

Probably all cases of trachoma have commenced with the formation of small granules. The majority of adults with classical trachomatous lids state that they have had the complaint 'ever since childhood.'

The disease is often so insidious in its onset, and its symptoms so slight, that a whole school may be affected without any suspicion of its presence until a few children have discharge from the eyes.

As a result of this insidious onset many cases are not seen until the disease is well advanced and the granules large. It is sometimes difficult to obtain a history long enough to account for the presence of large granules. They occasionally grow remarkably quickly and form mushroom-like elevations on the conjunctiva similar to the warty growths of gonorrhoea in the urethra.

TABLE I

CATARRHAL OPHTHALMIA WITH SMALL GRANULES ON THE UPPER
CONJUNCTIVA (FOLLICULAR CONJUNCTIVITIS)

Case	Age	Sex	Amount of Discharge	Organisms Isolated
1	2½ years	Male	Scanty	Koch-Weeks bacillus. Xerosis bacillus.
2	5½ years	Male	Scanty	Koch-Weeks bacillus. Xerosis bacillus.
3	6 weeks	Female	Very scanty	Koch-Weeks bacillus. Xerosis bacillus. Staphylococcus epidermidis albus.
4	5 years	Male	Small	Koch-Weeks bacillus. Xerosis bacillus. Staphylococcus epidermidis albus.
5	3 years	Female	None	One or two slender bacilli seen. Xerosis bacillus. Streptococcus brevis. Staphylococcus epidermidis albus.
6	3 years	Female	Small	Koch-Weeks bacillus. Xerosis bacillus. Pseudo-diphtheria bacillus.
7	21 mths.	Female	Small	Koch-Weeks and xerosis bacillus. No. 1, Table III (Part I).
8	21 mths.	Male	Small	Koch-Weeks bacillus. No. 1, Table III (Part I).
9	5½ years	Female	Large	Koch-Weeks bacillus and xerosis bacillus.
10	13 years	Female	Small	Koch-Weeks and xerosis bacillus. Streptococcus longus. Sarcina lutea. Pseudo-diphtheria bacillus.
11	24 years	Female	Small	Koch-Weeks and xerosis bacillus.
12	5 mths.	Male	Small	Koch-Weeks and xerosis bacillus.
13	3½ years	Male	Small	Koch-Weeks bacillus and xerosis bacillus
14	5 years	Female	Scanty	Koch-Weeks and xerosis bacillus. Staphylococcus pyogenes albus.
15	3 years	Female	Small	Bacillus lacunatus (Eyre). Staphylococcus pyogenes albus. Xerosis bacillus (in enormous numbers). One or two slender bacilli.
16	Child	Female	Small	Pneumococcus. Streptococcus brevis.
17	11 years	Female	Scanty	Xerosis bacillus. Staphylococcus pyogenes aureus and citreus.
18	9 years	Female	None	Bacillus capsulatus mucosus. Bacillus coli communis.
19	3 years	Male	None	Xerosis bacillus. Staphylococcus citreus.
20	2 years	Female	None	Koch-Weeks bacillus. No. 3, Table IV. Xerosis bacillus.

In catarrhal ophthalmia with some small granulations on the upper lid, Koch-Weeks bacillus is almost a constant factor in the discharge.

A glance at the table will show that out of a series of twenty observations Koch-Weeks bacillus occurred in fourteen in the discharge, and was doubtful in a fifteenth.

The first five cases are from a series of six which were left for a month without treatment, to see if residence in the country with plenty of fresh air and good food had any influence in causing the disappearance of the small miliary granules.

It was then found that instead of having diminished in size, the granules had undergone a very appreciable enlargement, and the whole of the upper palpebral

conjunctiva was studded with pale pinkish elevations. The discharge in each case was not abundant, and when present appeared as one or two small pellicles in the upper and lower fornix conjunctivae.

In the fifth case, where there was no discharge, one or two slender bacilli were seen in films of the lachrymal fluid.

The sixth case of this series had large granules, and has been placed in the second sub-division.

Cases 15 and 16 were complicated by the presence of *B. lacunatus* (EYRE) and the pneumo-coccus, and the inflammation was probably due to their presence. In one of them, one or two slender bacilli were detected in the discharge.

In 17, 18, and 19, there was practically no discharge, and films were not made. The four last cases had been for about a week under treatment, and the inflammatory process had been to some extent arrested, yet in the last case careful examination of a little discharge showed one or two slender bacilli.

The organism that occurred most frequently was the xerosis bacillus. This bacillus is found so often in healthy and diseased conditions that it must be considered a regular inhabitant of the conjunctival sac.

Its repeated occurrence in all forms of granular lids made it necessary to prove that it could have no action in causing the granular condition.

With this object in view various experiments were performed on the conjunctiva of rabbits.

1. A pure culture was emulsified in sterile broth, and about 0.25 c.c. inoculated beneath the conjunctiva. On the next day the fluid was absorbed, and subsequently, with the exception of a little redness about the point of inoculation, there was no inflammatory manifestation.
2. Together with two loopfuls of the pure culture a little finely-powdered glass was rubbed gently over the conjunctiva: slightly momentary irritation ensued, which quickly subsided. In twenty-four hours the appearance was normal and there was no congestion.
3. Every day for fourteen days one loopful of a one day old serum culture of the xerosis bacillus was gently smeared over the conjunctiva. On the next day after each inoculation the conjunctiva was normal, and at the end of fourteen days or subsequently there was no appearance of granules.

Additional evidence against the probability of xerosis bacillus being in any way responsible for granular lids is gathered from its occurrence in all diseased states of the conjunctiva. In xerosis of the conjunctiva, in which it was first described by KUSCHBERT and NEISSER, it is present in enormous numbers; and one small loopful of lachrymal fluid from the healthy conjunctiva has been found to contain over two hundred bacilli.

An interesting fact observed many times, is that, with approaching convalescence from inflammatory states, this bacillus is found sometimes in the discharge in very large numbers.

A striking instance of this was seen in an infant recovering from muco-purulent catarrh, whose mother (case 11) suffered from granular lids. When first examined the discharge consisted mainly of fibrin, and contained very few organisms. Seven days afterwards the small amount of whitish discharge which was obtained practically consisted of xerosis bacilli.

In one case of granular lids a typical appearance of xerosis of the conjunctiva was noticed.

The part the bacillus plays in the several processes of the eye has yet to be determined, but the observation is well-founded that the diminution of inflammation seems to be the signal for the growth of the xerosis bacillus.

The other organisms found in catarrhal ophthalmia with small granules were not constant in their incidence, and occurred in the healthy eye and in other inflammatory conditions.

TABLE II
GRANULES LARGE (TRUE TRACHOMA)

No.	Age	Sex	Amount of Discharge	Organisms
1	2½ years	Female	Large	Koch-Weeks bacillus. Staphylococcus albus and aureus
2	...	Female	Large	Koch-Weeks bacillus
3	13 years	Male	None	One or two slender bacilli. B. xerosis
4	...	Female	Small	One or two slender bacilli. B. xerosis. Staphylococcus pyogenes albus
5	2 years	Male	Scanty	B. xerosis. Staphylococcus epidermidis albus. No. 3, Table IV (Part I)
6	21 mths.	Male	Small	B. lacunatus (Eyre). Staphylococcus pyogenes albus and citreus. No. 3, Table IV
7	7 years	Male	Large	B. lacunatus (Eyre). B. xerosis. Staphylococcus pyogenes aureus and albus
8	34 years	Female	Small	Pseudo-diphtheria bacillus (No. 5, Table IV)
9	16 years	Female	Scanty	B. xerosis. Staphylococcus pyogenes albus
10	12 years	Female	Scanty	B. xerosis. Sarcina lutea. Streptococcus brevis
11	21 years	Male	None	B. xerosis (enormous numbers)
12	2½ years	Male	Scanty	B. xerosis. Staphylococcus epidermidis albus

In the first two cases, where the discharge was considerable, Koch-Weeks bacillus was observed in quantity.

In three and four, where there was no discharge, one or two slender bacilli were observed after examining a large field. It was only possible to say that these slender bacilli resembled exactly the morphological appearance of Koch-Weeks bacillus; in cases where the bacillus was with difficulty found no cultures could be obtained.

Case five belongs to the series of six which was kept for a month under observation. The discharge in this case was almost wholly fibrin, only a few leucocytes and epithelial cells being seen; in films of the discharge no organism could be detected.

Case eight was examined four times at different periods in six months, and on every occasion a pseudo-diphtheria bacillus was obtained in pure culture; its characters are given in Table IV (No. 5). This was the only occasion on which this variety of pseudo-diphtheria bacillus was isolated.

A glance at Table II (page 135) shows that no organism occurs with sufficient constancy to justify a causal connection with granular lids.

Also each organism isolated was inoculated on the conjunctiva of a rabbit and in no case did a granular condition result.

TABLE III

CHRONIC TRACHOMA WITH CICATRICIAL THICKENING

No.	Age	Sex	Condition of Lids	Organisms
1	15 years	Female	Some large granules with cicatricial thickening of upper lid	Staphylococcus aureus. Xerosis bacillus.
2	Adult	Female	Entropion. Opacity of cornea	B. lacunatus (Eyre) and xerosis. Staphylococcus aureus and epidermidis albus.
3	Adult	Female	Pannus. Large granulations and thickening of upper lid	B. xerosis. Staphylococcus epidermidis albus.
4	Adult	Female	Ectropion. Conjunctiva red, velvety, and thickened	Streptococcus longus. Staphylococcus citreus. B. xerosis.
5	17 years	Male	Thickening and distortion of upper lid. Opacities of cornea. Obliteration of upper and lower fornices with strong fibrous bands	Fraenkel's pneumococcus. B. lacunatus and xerosis. Staphylococcus aureus and citreus.
6	25 years	Male	Upper lid thickened. Conjunctival surface smooth and whitish. Adherence of ocular and palpebral conjunctiva, causing contraction of the conjunctival sac	Xerosis bacillus.
7	20 years	Male	Ectropion. Conjunctiva hypertrophied, red, and velvety	Staphylococcus aureus. Xerosis bacillus.
8	Adult	Male	Conjunctiva velvety and thickened. Old opacities of cornea	Xerosis bacillus.
9	Adult	Female	Cicatricial distortion. A few large granules	Streptococcus longus. Xerosis bacillus.
10	Adult	Female	Upper lid very thickened. Conjunctival surface smooth and whitish	Xerosis bacillus.
11	26 years	Male	Upper lid thickened. Conjunctiva hypertrophied, red, and velvety	Xerosis bacillus. Staphylococcus aureus.
12	14 years	Male	Entropion. Fibrous adhesions between ocular and palpebral conjunctivae. Some large granules	B. lacunatus (Eyre). Staphylococcus pyogenes albus and citreus. Sarcina lutea. Xerosis bacillus.
13	Adult	Female	Entropion. Conjunctiva covered with large irregular granules. Fibrous adhesions between the ocular and palpebral conjunctiva. Extreme pannus	Streptococcus pyogenes longus. Staphylococcus epidermidis albus.

With scarcely one exception the disease in Table III was of considerable duration ; most of the patients could give no definite history, with the exception that they had had 'sore eyes' since childhood. It would, therefore, not be expected that in such a series uniformity in the incidence of a specific organism would be secured, and in the consideration of the agents causing trachoma this table has not been taken into account.

The pyogenic cocci were of frequent occurrence ; *staphylococcus aureus* was found five times ; *staphylococcus citreus*, three times ; *staphylococcus albus*, once ; *streptococcus longus*, three times ; *pneumococcus*, once.

B. lacunatus (EYRE) was isolated on three occasions, once associated with FRAENKEL'S *pneumococcus*. On each occasion there was considerable inflammatory reaction, and it is probable that the inflammation caused by the bacillus was super-added to the granular lids. *B. lacunatus* rarely causes an acute conjunctivitis, but the conditions for its growth are much more favourable in a conjunctiva disorganized by granules than in a previously normal one. Xerosis bacillus occurred in every case, in some of them in large quantity.

In two of the cases, 6 and 10, where all sign of granules had disappeared and the conjunctiva had undergone natural recovery, xerosis bacillus was obtained in pure culture. *Staphylococcus aureus* was almost invariably found when the conjunctiva was thickened and velvety.

The almost constant appearance of Koch-Weeks bacillus in the first group, makes the conclusion inevitable that this bacillus is very often the cause of an inflammation of the conjunctiva, accompanied by the formation of granules. It has been seen that when these cases have been left untreated for some time the granules grow in diameter. Also, not only have cases been observed to develop granular lids from simple muco-purulent catarrh, but also several families have come under observation, in which the individuals have each suffered from muco-purulent catarrh, or some form of granular lids, the cause of the muco-purulent catarrh being in all cases Koch-Weeks bacillus.

These facts are strong evidence that the different varieties of granular lids are stages of the same disease, and that in all probability all cases of trachoma have commenced in the formation of the minute miliary granules, as a result of infection by Koch-Weeks bacillus.

The view is now generally accepted that trachoma is a specifically contagious disease due to some organism. In epidemics of trachoma in schools, muco-purulent catarrh often accompanies and equals trachoma in incidence.

The failure to find Koch-Weeks bacillus in more than four cases (? two) of the second group may be accounted for by the fact that—

- (a) Several had lasted over a year.
- (b) Discharge was scanty or even absent.
- (c) Treatment had been given in many cases.
- (d) The disease was complicated by infection by *B. lacunatus* (EYRE) in two cases.

Moreover, when the discharge is very scanty, one or two isolated slender organisms cannot be definitely stated to be Koch-Weeks bacillus, and attempts to obtain cultures under these circumstances are usually unsuccessful. It is also extremely probable that, a granular condition having been set up by the Koch-Weeks bacillus, slight forms of irritation, microbial or otherwise, which would have little effect on the normal conjunctiva, are sufficient not only to cause a continuance of the inflammation, but also to produce an actual increase in the size of the granules.

Additional support to the view that Koch-Weeks bacillus is the causal agent in trachoma, is afforded by—

- (a) The association of muco-purulent catarrh and granular lids in epidemics of ophthalmia in Egypt.
- (b) The observations of many writers, that inflammations of the conjunctiva caused by the Koch-Weeks bacillus often leave behind a granular condition.

HISTOLOGY OF THE TRACHOMA GRANULE

In a former paper contributed to the Thompson Yates Laboratory Reports, vol. ii, 1900, I have described the histological characters of trachomatous lids.

The granules were found to be chronic inflammatory nodules, consisting of small round cells and a delicate reticulum of fibrous tissue enclosed by an incomplete capsule of bands of fibrous tissue.

Examples of the different stages in the formation of a granule were obtained. Sections of an acutely inflamed conjunctiva showed a general infiltration of the tissue with small round cells and here and there small circumscribed masses of cells which did not possess a capsule. In sections of an inflamed conjunctiva in which there were small granules, the round cell masses were more distinctly circumscribed and, although not possessing a capsule, had a well-defined reticulum of fibrous tissue. A later development is the formation of a fibrous capsule and evident signs of cicatrization of the nodule. In all the cases plasma cells were abundant, and several appeared to have ruptured and discharged their granules into the surrounding tissue.

With regard to the presence of organisms in the sections, no very positive result was obtained, but in one or two instances bodies that had a great resemblance to the slender bacilli were observed; these were especially noted in a cover-glass preparation of a crushed follicle.

PREVIOUS LITERATURE

SHONGOLOWICZ³⁹ considers the importance of examining the tissues as well as the discharge for organisms. In the crushed contents of follicles he found very small short rods which stained badly and chiefly at the poles. They grew on all media, but poorly; they grew best on flesh peptone-agar, producing a greenish colour.

Inoculation of rabbits and cats produced a certain similarity to trachoma in man.

SHONGOLOWICZ does not doubt that he has found the true cause of trachoma, and ascribes the discovery of cocci by SATTLER to the difficulty of staining the organism, and the fact that it stains bipolarly. (It is not unlikely that this organism staining bipolarly was the xerosis bacillus).

In twenty-six cases of trachoma he found staphylococcus albus in twelve; staphylococcus citreus in three; staphylococcus aureus in nine; staphylococcus cereus albus in three; and in seven cases the short bacillus.

MULLER,³⁴ from the secretion in trachoma disease, obtained a slender bacillus, which resembled morphologically and culturally the influenza bacillus. In fifteen cases he found the bacillus eleven times. He further remarks that he found the bacillus in old cicatricial trachoma only when there was a certain amount of discharge.

The description of the bacillus would apply to the Koch-Weeks bacillus, and in this respect his observations agree with mine.

LOGETSCHNIKOW³⁰ thinks that follicular catarrh and trachoma are not one and the same, their identity has yet to be proven. He thinks that the micro-coccus of Michel should be more appropriately designated as the coccus of follicular catarrh instead of trachom-coccus.

SCHMIDT,³⁸ in forty-seven out of fifty-eight cases, failed to obtain cultures of the trachom-coccus, which he found to possess great morphological resemblance to the staphylococcus pyogenes. Inoculation produced a severe muco-purulent conjunctivitis in dogs, rats, and rabbits. In pigeons, he states that the cocci produced typical trachoma.

STERNBERG⁴³ remarks that the description of the trachom-coccus would apply to some of the more common pus cocci, *e.g.* Staphylococcus pyogenes aureus, which have also been shown to consist of two hemispherical halves separated by a narrow line of sub-division.

KARTULIS failed to find SATTLER's trachom-coccus or any other organism in the contents of a trachomatous follicle. He states that if treatment be neglected in muco-purulent catarrh caused by Koch-Weeks bacillus, a granular infiltration of the conjunctiva results, which subsequently offers the clinical picture of trachoma.

WILBRAND, SAENGER, and STAELIN⁴⁴ observed that in conjunctivitis caused by Koch-Weeks bacillus changes remained behind which suggested true trachoma, but they add that in these cases follicle formation has existed from the beginning.

HOFFMANN²³ states that cases which have hitherto been taken for papillary trachoma are cases of conjunctivitis caused by Koch-Weeks bacillus which have become chronic.

REICH³⁷ calls attention to the fact that although follicles of not specifically trachomatous character may be observed, we are unable to distinguish them from the milder forms of trachoma.

FULTON¹¹ regards follicular conjunctivitis and trachoma as pathologically the same disease and believes in the direct contagiousness of both.

In COPPEZ⁴ opinion follicular and granular conjunctivitis are identical.

ZIEGLER⁴⁸ observes that follicular catarrh and trachoma are indistinguishable, but that follicular catarrh never quite produces the cicatricial degeneration seen in true trachoma. He further states that follicular catarrh can be produced by atropine and that it is simply the expression of a chronic irritation ; also that it seems to be originated by a diplococcus resembling the gonococcus, but which stains by GRAM.

LEBER²⁹ describes the presence of certain large cells containing peculiarly formed bodies in trachoma, and he thinks that these have probably to do with the pathogenesis of the affection.

GERMANN¹² in three cases of acute trachoma states that the infection had apparently been brought about by black earth in the conjunctival sac. He considers that the distribution of trachoma is effected by the dust of the fields and agricultural employments in which much dust is engendered.

HIRSCH²⁰ and HIRSCHBERG²¹ give some points on the distribution of trachoma in certain districts of Europe. TRUC suggests methods for the prevention of the distribution of the affection. In their works no bacteriological or histological examinations were made.

HERBERT¹⁹ states that in trachoma and conjunctivitis the formation of adenoid tissue from connective tissue can be traced, and that in follicular and granular conjunctivitis the new formations might differ from the normal in being deficient in supporting stroma and blood vessels.

Professor GUARNIERI¹⁷ describes some bodies, staining intensely with magenta red, one-third to one-half the size of red blood corpuscles, which he obtained after grattage of trachomatous lids. He suspects that they are of a parasitic nature and belong to the blastomycetes ; he did not succeed in cultivating them.

PICK³⁶ states that—

- (a) The follicles are not immediately contiguous to the epithelium, but are separated from it by bands of fibrous tissue circularly arranged around the nodule.
- (b) The follicle cells are of the same kind as the round cells found in the conjunctival stroma.
- (c) The trachoma bodies of Burchardt are found only in the epithelium and have absolutely nothing to do with protozoa.

STEPHENSON⁴¹ states that muco-purulent catarrh does not last long, and even when not subjected to treatment does not produce any serious or permanent lesion of the eye. He says that the most important forms of disease which are prevalent in schools are follicular ophthalmia and trachoma. The supposed transition of follicular disease into trachoma has arisen, it is thought, from children with follicular disease being placed in wards with trachomatous children from whom they have contracted that infectious disease. Very few instances of trachoma occurred in children under two years of age.

IX. DIPLO-BACILLARY CONJUNCTIVITIS

In the out-patient department of the Royal Infirmary a number of patients applying for treatment for refractive errors was met with, who, on enquiry, gave a history of a little discharge in the morning 'gumming' the lids and sensations of burning or grit in the eyes.

No definite history as to duration could be obtained; they had all suffered from morning discharge for some time. Twelve cases have been investigated; the patients were all middle-aged women and at the time of observation the disease was symmetrical.

When the lids were everted the conjunctiva was seen to be congested and slightly velvety, but even in the most protracted case there was no cicatricial distortion or thickening of the eyelids.

In some of the cases there was a small pellicle of white muco-pus floating in the lachrymal fluid, and perhaps a little dried secretion around the roots of the eyelashes.

In films of the discharge the organism most frequently observed was a short thick bacillus, commonly seen in pairs, but also in short chains of eight or more elements.

Cultures on Serum. After twenty-four's incubation at 38° C. the surface of the serum was eroded with numerous shallow pits which, on further incubation, grew rapidly in depth and extent, liquefying the serum.

At first no growth was visible, but later a little greyish growth appeared at the bottom of the pits.

Film preparations of this growth showed a short thick bacillus; involution forms were early and exceedingly irregular in shape.

In nine out of twelve cases this bacillus was cultivated.

ANALYSIS OF TWELVE CASES

Case	Organisms Isolated
1 ...	<i>B. lacunatus</i> (EYRE).
2 ...	<i>B. lacunatus</i> .

Case	Organisms Isolated			
3	...	B. lacunatus.	Staphylococcus aureus.	B. xerosis. Pseudo-diphtheria bacillus.
4	...	B. lacunatus.		
5	...	B. lacunatus.		
6	...	B. lacunatus.		
7	...	B. lacunatus.	Staphylococcus epidermidis albus and aureus.	B. xerosis.
8	...	B. lacunatus.	Staphylococcus aureus.	
9	...	B. lacunatus.	B. xerosis.	
10	...	Staphylococcus albus and epidermidis albus.	B. xerosis.	
11	...	B. xerosis.		
12	...	B. xerosis.	Staphylococcus albus.	

The bacillus has been met with frequently in children, but always complicating granular lids ; it was isolated nine times from conjunctivae apparently healthy. On a few occasions a bacillus closely resembling it has been observed in blepharitis in the discharge around the eyelashes.

BIARD³ states that the diplo-bacillus is never present in the normal conjunctival sac, but is a constant inhabitant of the nasal fossa, and he concludes that infection takes place either by direct extension or by transference by means of the fingers.

MORAX³² and AXENFELD² simultaneously and independently described a diplo-bacillus which they isolated from subacute cases of conjunctivitis. AXENFELD also found it in acute cases. EYRE⁸ found the bacillus in many cases of conjunctivitis in which the chronicity of the inflammation and the contagious nature of the discharge were marked. He states that both sexes, and all ages, are susceptible, but that it is most common in middle-aged women. The objective signs were slight, being confined to a little mucus-like discharge adhering to the roots of the eyelashes and collecting in the neighbourhood of the caruncle, also slight injection of the bulbar conjunctiva, and marked injection of the palpebral conjunctiva with an erythematous fringe along the free edge of the lower lid.

X. AFFECTIONS OF THE LACHRYMAL SAC

Only five cases of swelling of the lachrymal sac have been met with. Two were cases of mucocele in which the contents were clear and viscid ; three were cases of acute purulent dacryocystitis.

In brief, the results of the bacteriological examination were as follows :—

MUCOCELE	
Case	
1	Koch-Weeks bacillus. Staphylococcus epidermidis albus.
2	Koch-Weeks bacillus. Staphylococcus pyogenes albus. Pseudo-diphtheria bacillus.

ACUTE DACRYOCYSTITIS

Case

- 1 Girl, aged 13. *Streptococcus pyogenes longus*.
- 2 Girl, aged 10. *Streptococcus pyogenes*. *B. xerosis* and pseudo-diphtheria bacillus. *Staphylococcus citreus*. White cladothrix.
- 3 Girl *Streptococcus pyogenes*.

The streptococcus from case 1 was inoculated in a mouse, which died in five days from general infection.

These observations confirm the results of MORAX, PARINAUD³⁵, and EYRE⁷.

MORAX found Koch-Weeks bacillus in a large number of his cases of mucocele.

MORAX and PARINAUD isolated streptococcus pyogenes from the pus of acute inflammation of the lachrymal sac. EYRE investigated twenty-six sac cases. In ten cases of acute purulent dacryocystitis streptococcus was constant, and in a large proportion of the chronic cases streptococcus occurred. In six cases of mucocele Weeks bacillus was isolated three times.

UNCLASSIFIED CASES

In all about two hundred individuals with inflamed eyes have been examined; in many, discharge has been collected from both eyes. A large number of the cases has not been classified; they include, mainly, simple inflammatory states that have been for some time under treatment, chronic inflammatory states with no definite history, traumatic cases, etc.

A few cases of blepharitis and ulcer of the cornea have been examined, but the number has not been sufficient for any definite conclusions to be arrived at. In blepharitis staphylococcus aureus frequently occurred, and in some cases a short bacillus was obtained, which closely resembled the diplo-bacillus of MORAX.

In one case of ulcer, with bulging of the cornea, a little of the discharge removed from the surface of the cornea was found to contain remarkably few organisms of any kind: cultures of *B. lacunatus* (EYRE); staphylococcus epidermidis albus; *B. xerosis* and a pseudo-diphtheritic bacillus were obtained.

In a case of membranous conjunctivitis, closely resembling the conjunctivitis produced by *B. diphtheriae*, only *B. xerosis* and a pseudo-diphtheritic bacillus were isolated. Inoculation of a large quantity of these bacilli into guinea-pigs produced no reaction.

A pure case of pneumococcus ophthalmia was not met with ; on the one occasion on which it occurred it was complicating cicatricial trachoma, and was associated with *B. lacunatus* (EYRE). Inoculation of a pure culture into a mouse showed that it had lost its virulence.

Two cases of epithelial xerosis of the conjunctiva, one associated with night blindness and no evident disease of the palpebral conjunctiva, the other associated with granular lids, were investigated. The white flakes removed from the conjunctiva practically consisted of the xerosis bacillus.

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BILE SALT BROTH

BILE SALT BROTH

I. A SIMPLE TEST FOR FAECAL CONTAMINATION

II. THE BEHAVIOUR IN BILE SALT BROTH, IN CERTAIN SUGARS, AND IN GLYCERINE, OF SOME OF THE COMMONER ORGAN- ISMS—WITH SPECIAL REFERENCE TO THE EFFECT OF THEIR PRESENCE UPON THE VALUE OF THE ABOVE TEST

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AND

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ASSISTANT BACTERIOLOGISTS TO THE ROYAL COMMISSION ON SEWAGE DISPOSAL

I

In a previous number of these reports (*T. Y. Reports*, vol. iii, part 2) one of us (MACCONKEY) described a medium which gives a most characteristic reaction when inoculated with *B. coli communis* or *B. typhi abdominalis*. This is an agar medium containing taurocholate of sodium and lactose. Upon this the colonies of B.C.C. grow freely and rapidly; they assume a yellow colour and give rise to a fine precipitate in their vicinity—thus producing the appearance of a halo—on the other hand the colonies of B.T.A., though not usually apparent at the end of twenty-four hours, are after forty-eight hours plainly visible as whitish translucent roundish colonies without any surrounding haze. Further it was found that the medium was unfavourable to the growth of all the commoner forms of spore-bearing organisms—such as *B. subtilis* and its allies—which are usually present in water, whilst the temperature of incubation ~~41.2~~ 42°C. further inhibited the growth of most of the ordinary water bacteria.

In order to simplify this test further—by the use of a fluid instead of a solid medium—a taurocholate glucose broth has been devised.

The composition of the medium is as follows :—

Sodium Taurocholate	0.5 per cent.
Glucose	0.5 „
Peptone	2.0 „
Water	100 cc.

These constituents are heated together until the solids are dissolved and then filtered. After filtration, sufficient neutral litmus solution to give a distinct colour is added. The medium is then distributed into test tubes, and one of DURHAM's fermentation tubes placed in each. Sterilization is effected in twenty minutes by KOCH's sterilizer on each of three successive days. After the third sterilization the small inner tube should be quite full. Glucose was used instead of lactose in order not to exclude the *B. enteritidis* of GAERTNER and allied organisms, as they are incapable of fermenting lactose.

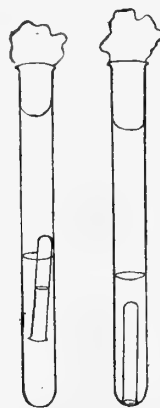
The percentage of glucose was determined by careful experiments, which led to the conclusion that any higher percentage was detrimental to the growth of the B.C.C. and allied organisms.

The absence of sodium chloride and the extractives of meat enables the medium to be of an almost uniform composition, of which the advantage is obvious. The temperature of incubation is 42°C ., the same as that for the agar medium ; this enables the reaction to occur quickly and favours the growth of all intestinal organisms. The litmus indicates at once whether there is acid formation. The DURHAM's tube shows whether there is fermentation of the glucose.

The taurocholate favours the growth of the group of organisms which are the most important from the point of view of faecal contamination, viz., the B.C.C. and GAERTNER groups, etc.

CHARACTERISTIC REACTION

On inoculating this medium with B.C.C. or the GAERTNER bacillus, the following characteristic reaction is produced within forty-eight hours. The appended drawings show it clearly.



The medium is uniformly red in colour. The fermentation tube is filled with gas, and the surface of the medium is covered over with a mesh work of bubbles, whilst a constant stream of bubbles can be seen rising to the surface. After inoculation with B.T.A., the medium becomes uniformly red in colour, but there is no production of gas as the bacillus is incapable of fermenting glucose.

TIME OF INCUBATION

In order to make the test as rapid as possible, it was, at the outset, necessary to determine some limit for the time of incubation. This we have fixed at forty-eight hours, as we are satisfied, from numerous experiments, that if B.C.C., etc., be present, the reaction occurs within this limit. In the majority of cases, it is within twenty-four hours.

PRECAUTIONS TO BE OBSERVED WHEN USING THE TEST

1. *Formation of Gas.* Frequently a few small bubbles collect in the upper part of the fermentation tube after incubation. This is apparently due to evaporation of liquid from the tube owing to the high temperature, and can readily be recognized. It is totally different in appearance to the fermentation caused by the reacting organisms, which is general: the whole liquid being permeated with rising bubbles of gas, and the surface covered with a meshwork of the same.

2. *Pipettes.* It is advisable to use a separate pipette for making each dilution, and for inoculating the tubes; but if this be impracticable, one pipette should be used for making the dilution, and a second for inoculating the tubes, care being taken to commence the inoculation at the highest dilution.

3. *Plates.* When it is desired to make plate cultivations from the Taurocholate broth, this should be done forty-eight hours after inoculation, as the development of acid tends to kill off the organisms.

We have examined the behaviour in this medium of ninety-four organisms, most of which were obtained from Král's laboratory.

GROUPING OF ORGANISMS

Four headings obviously present themselves under which the organisms can be grouped:—

- I. Those producing acid and gas.
- II. Those producing acid only and no gas.
- III. Those capable of growth in the medium, but producing neither acid nor gas.
- IV. Those incapable of growth in the medium.

and finally a fifth group:

- V. Those incapable of growth on any medium at the temperature of incubation, viz., 42° C.

Division I contains seventeen organisms:—

1. *B. coli communis* (ESCHERICH) (subculture of original strain)
2. *B. acidi lactici* (HUEPPE)
3. *B. cavidida* (BRIEGER)

4. *B. coli communis* (Laboratory stock culture)
5. *B. neapolitanus*
6. *B. capsulatus* (PFEIFFER)
7. *B. lactis aerogenes* (ESCHERICH) (subculture of original strain)
8. *B. enteritidis* (GAERTNER)
9. *B. icteroides* (SANARELLI) (subculture of original strain)
10. *B. paracolon* (LE SAGE)
11. *B. of epidemic jaundice*
12. *B. psittacosis* (NOCARD)
13. *B. of hog cholera* (SALMON and SMITH)
14. *B. pneumoniae* (FRIEDLÄNDER)
15. *B. der frettchenseuche* (EBERTH)
16. *B. cloacae* (JORDAN)
17. *M. candicans* (FLÜGGE)

Division II contains twenty-two organisms :—

18. *B. typhosus*
19. *B. pyogenes foetidus*
20. *B. of dysentery* (KRUSE)
21. " (FLEXNER)
22. " (SHIGA)
23. " (GRAY)
24. " (HARRIS)
25. *Vibrio cholerae* (BERLIN)
26. " " (MARSEILLES)
27. " " (ELVERS)
28. " " (FROHNER)
29. " " (ARGEL)
30. " *Metschnikovi*
31. *B. rhinoscleroma*
32. *Proteus vulgaris*
33. *B. prodigiosus*
34. *B. pseudo-tuberculosis* (PFEIFFER)
35. *B. der Darm-Diphtherie* (RIBBERT)
36. *B. fluorescens liquefaciens* (FLÜGGE)
37. *Staphylococcus pyogenes aureus*
38. " " *albus*
39. " " *citreus*

Division III contains fifteen organisms :—

40. *B. butyricus* (HUEPPE)
41. *B. butyricus*
42. *B. filamentosus*
43. *B. buccalis maximus*
44. *B. mesentericus vulgatus*
45. *B. mesentericus fuscus*
46. *B. mesentericus niger*
47. *B. pyocyaneus*
48. *B. Zopfi*
49. *B. disciformans* (ZOPF)
50. *B. brunneus*
51. *B. megatherium*
52. *V. Finkler-Prior*
53. *M. ureae*
54. *Sarcina lutea*

Division IV contains nineteen organisms :—

55. *B. anthracis*
56. *B. anthracoides*
57. *B. diphtheriae*
58. *B. faecalis alkaligenes*
59. *B. fluorescens albus*
60. *B. fluorescens putidus*
61. *B. necro-dentalis*
62. *B. Stutzeri*
63. *B. subtilis*
64. *B. xerosis*
65. *M. tetragenus*
66. *Sarcina aurantiaca* (FLÜGGE)
67. *Sarcina flava* (DE BARY)
68. *Sarcina ventriculi*
69. *Saccharomyces ellipsoideus*
70. *Cladothrix dichotoma*
71. *Torula alba*
72. *Torula rubra*
73. *Streptococcus pyogenes*

Division V contains twenty-one organisms :—

74. *B. arborescens*
75. *B. aureus*
76. *B. candicans*

77. *B. diffusus*
78. *B. fluorescens aureus*
79. *B. fluorescens mesentericus*
80. *B. fuscus*
81. *B. gelber* (KORN)
82. *M. mycoides*
83. *B. proteus mirabilis*
84. *B. proteus Zenkeri*
85. *B. ramosus*
86. *B. of haemorrhagic septicaemia*
87. *M. agilis*
88. *Oidium lactis*
89. *Spirillum rubrum*
90. *Saccharomyces cerevisiae* I
91. *Saccharomyces urinae*
92. *B. pestis*
93. *St. cereus albus*
94. *B. Violaceus*

On examining the list of organisms contained in groups I and II, it will be seen that the large majority are intestinal in origin. Those of group I, viz., those producing the reaction of gas and acid formation, are with one or two exceptions obviously intestinal organisms. *It is, therefore, justifiable to conclude, that when the reaction is obtained, it is most probably produced by organisms of intestinal origin.*

When an incomplete reaction is obtained, viz., acid formation only and no gas, there is still strong suspicion of intestinal pollution, as it will be seen most of the organisms in group II (including Typhoid, Dysentery, and Cholera) are of the same origin. *Conversely it may be stated, that when the reaction is not present faecal contamination is absent.*

PRACTICAL APPLICATION OF THE TEST

To prove the practicability of this test, samples of drinking and river water sent to the Laboratory have been tested by this method. Similarly, various dilutions of crude sewage and sewage effluents have been subjected to this fermentation test. Comparative tests with the taurocholate agar have in many instances been made side by side.

METHOD

In the case of drinking water, 1 cc. of the water is added to the broth in each of three separate tubes respectively, the three tubes are incubated at 42° C. for forty-eight hours, and the results read off.

In the case of crude sewage, effluents, or river water, definite dilutions of the cubic centimetre are made in each instance, viz., $\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1,000}$, $\frac{1}{10,000}$, $\frac{1}{100,000}$, according as it is desired to determine whether there are 10, 100, 1,000, 10,000, 100,000 B. coli present in the cubic centimetre of the sample (other dilutions can also be made as occasion demands). Four distinct dilutions are severally tested, and three determinations made of each dilution. Thus, twelve tubes of the medium are used for each sample. The tubes are incubated at 42° C. for forty-eight hours, as in the previous instance.

It is found in practice that in the large majority of cases the characteristic reaction is obtained within twenty-four hours, frequently within eighteen hours. In the case of the higher dilutions more time seems to be requisite, so that it is advisable to incubate for forty-eight hours. If the reaction has not occurred within that time, it can be definitely stated that further incubation will not produce it. The medium may become acid or slowly decolourized, but the formation of gas is never observed; when it occurs, it takes place rapidly.

RESULTS

A. *Drinking water*—1. *Moorland filtered water*:

Samples	118
Number of reactions	5
„ „ due to B.C.C.	5

2. *River filtered water*:

Samples	169
Number of reactions	16
„ „ due to B.C.C.	16

B. *River water*:

Samples	41
Number of reactions	41
„ „ due to B.C.C.	41

C. *Milk*:

Samples	103
Number of reactions	98
„ „ due to B.C.C.	98

D. *Sewage and sewage effluents*:

The reaction has always been obtained in every sample examined.

CONCLUSIONS

Above it has been stated that, considering the origin of the organisms that gave this reaction, it was justifiable to conclude that when reaction was obtained, faecal contamination was almost certainly present. The results obtained with waters and milk tend to confirm this conclusion.

We think, therefore, we are warranted in putting forward this test as a rapid means of detecting faecal contamination.

Organism	Tauro- cholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum	GLYCERINE	
									48 hrs.	6 days
1 <i>B. coli communis</i> (Escherich)	A and G	A and G	A and G	A and G	nil	A and C	Opaque-white, many with orange centre. Haze in depths	White, non-liquefying	D	A
2 <i>B. acid lactic</i> (Hueppe) (K)	A and G	A and G	A and G	A and G	nil	A and C	Opaque-white. Haze in depths	White, non-liquefying	D	A and G
3 <i>B. cavidia</i> (Brieger) (K)	A and G	A and G	A and G	A and G	nil	A and C	Round, flat, opaque white, some with orange centre. Haze	White, non-liquefying	D	A and D
4 <i>B. coli communis</i>	A and G	A and G	A and G	A and G	A and G	A and C	Opaque-white, many with orange centre. Haze	White, non-liquefying	D	D
5 <i>B. neapolitanus</i>	A and G	A and G	A and G	A and G	A and G	A and C	Like B.C.C. (Escherich)	White, non-liquefying	D	A and D
6 <i>B. capsulatus</i> (Pfeiffer) (K)	A and G	A and G	A and G	A and G	A and G	A and C	Surface: translucent, white, with clear ring around. Deep: orange, with haze Like B.C.C. (Escherich)	White, non-liquefying	A and G	...
7 <i>B. lactis aerogenes</i> (Escherich)	A and G	A and G	A and G	A and G	A and G	A and C	Flat, translucent, greyish white. No haze	White, non-liquefying	D	A and D
8 <i>B. enteritidis</i> (Gaertner)	A and G	A and G	D	A and G	D	A	Like B. enteritidis	White, non-liquefying	D	A, very slight
9 <i>B. Icteroides</i> (Sanarelli)	A and G	A and G	D	A and G	D	slight A	Like B. enteritidis	White, non-liquefying	D	A, very slight
10 <i>B. Para Colon</i> (Le Sage)	A and G	A and G	D	A and G	D	slight A	Like B. enteritidis	White, non-liquefying	D	A, very slight
11 <i>B. of epidemic jaun-</i> <i>dice</i>	A and G	A and G	D	A and G	D	A	Like B. enteritidis, but with filmy edge. No haze	White, non-liquefying	D	D
12 <i>B. psittacosis</i> (Nocard)	A and G	A and G	D	A and G	D	A	Like B. enteritidis, but with flat top and bevelled edge. No haze	White, non-liquefying	D	A and D
13 <i>B. of hog cholera</i> (Salmon & Smith)	A and G	A and G	D	A and G	D	A	Round, raised, opaque white. No haze	White, non-liquefying	D	Nch
14 <i>B. pneumoniae</i> (Friedländer)	A and G	A and G	nil (later A)	A and G	A and G	A	Round, raised, opaque, yellowish white. Haze	White, non-liquefying	D	A and G
15 <i>B. der Fretschenseuche</i> (Eberth) (K)	A and G	A and G	A	A and G	A and G	A	Opaque-white, mucoid. No haze	White, non-liquefying	Nch	A, very slight
16 <i>B. cloacae</i> (Jordan) (K)	A and G	A and G	A	A and G	A and G	(later C)	Opaque-white, mucoid. No haze	White, non-liquefying	Nch	A
17 <i>M. candidans</i> (Flügge) (K)	A and G	A and G	A	A and G	A and G	(later C)	Opaque-white, mucoid. No haze	White, non-liquefying	Nch	Nch

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization. (K) Obtained from Král's laboratory.

* Nos. 6, 7, and 14 ferment starch.

DIVISION II

Organism	Tauro- cholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum.	GLYCERINE	
									48 hrs.	6 days
18 <i>B. typhosus</i>	A	A	D	A	nil	nil or slight A	<i>Surface</i> : semi-transparent, greyish-white. <i>Deep</i> : opaque-white Like B.C.C. (Escherich)	Grey, non-liquefying	nil	A
19 <i>B. pyogenes</i> foetidus (K)	A	A	A	A	A	A and C		White, non-liquefying	nil	A and D
20 <i>B. dysentericus</i> (Kruse)	A	A	nil	nil	nil	nil	Flat, opalescent, very like B. enteritidis	White, non-liquefying	nil	A, very slight
21 <i>B. dysentericus</i> (Flexner)	A	A	nil	nil	nil	nil	" "	White, non-liquefying	nil	A, very slight
22 <i>B. dysentericus</i> (Shiga)	A	A	nil	nil	nil	nil	" "	White, non-liquefying	nil	A, very slight
23 <i>B. dysentericus</i> (Gray)	A	A	nil	A	nil	A	" "	White, non-liquefying	nil	A, very slight
24 <i>B. dysentericus</i> (Harris)	A	A	nil	nil	nil	A	" "	White, non-liquefying	nil	A, very slight
25 <i>V. cholerae</i> (Berlin) (K)	A	A	A	A	A	A	Flat, transparent, greyish- white. Slight haze	Pink, liquefying	nil	A
26 <i>V. cholerae</i> (Marseilles) (K)	A	A	A	A	A	A	" "	Pink, liquefying	nil	A
27 <i>V. cholerae</i> (Elvers)	A	A	A	A	A	A	" "	Pink, liquefying	nil	A
28 <i>V. cholerae</i> [(K) (Frohner) (K)	A	A	A	A	A	A	No growth	Pink, liquefying	nil	A
29 <i>V. cholerae</i> (Argel)	A	A	A	A	A	A	No growth	Pink, slight-liquefaction Scanty	nil	A
30 <i>V. Metchnikovi</i> [(K)	A	A	nil	A	A	slight A	No growth		nil	A
31 <i>B. Rhinoscleroma</i>	A	A	nil	nil	nil	A and C	<i>Surface</i> : Flat, greyish-white, translucent. <i>Deep</i> : like balls of wool, appearing hollow from below. No haze		nil	A
32 <i>Proteus vulgaris</i> (K)	A	A	D	nil	nil	slight A	Round, raised, translucent, greyish-white. No haze	Yellowish-white, non- liquefying	nil	A
33 <i>B. prodigiosus</i>	A	A	nil	A	A	A	No growth	Scanty	nil	nil
34 <i>B. pseudo-tuber- culosis</i> (Pfeiffer) (K)	A	A	nil	nil	nil	nil	No growth	Greyish-white, non- liquefying	nil	nil
35 <i>B. der Darm-</i> (K)	A	A	nil	nil	nil	nil	No growth	White, non-liquefying	nil	nil
36 <i>Diphtherie</i> (Ribbert) liquefaciens (Flügge)	A	A	nil	nil	slight A	A and C	No growth	Pink, slow-liquefaction	slight A	A
37 <i>St. pyogenes aureus</i>	A	A	A	nil	A	A and C	*No growth	Yellow, non-liquefying	nil	A
38 <i>St. pyogenes albus</i>	A	A	A	nil	A	A and C	*No growth	White, non-liquefying	nil	A, very slight
39 <i>St. pyogenes citreus</i>	A	A	nil	nil	A	slight A	*No growth	Yellow, non-liquefying	nil	A, very slight

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization (K) Obtained from Kral's laboratory.
* These organisms seem capable of growth on this medium, when freshly isolated.

DIVISION III

BILE SALT BROTH

161

Organism	Tauro- cholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum	GLYCERINE	
									48 hrs.	6 days
40 <i>B. butyricus</i> (Hueppe) (K)	Growth	A	A, very slight	slight A	slight A	slight A C P	No growth	Pinkish white, liquefying	nil	nil
41 <i>B. butyricus</i>	Growth	A	nil	nil	nil	A C P	No growth	White, non-liquefying	nil	nil
42 <i>B. filamentosus</i> (K)	Growth	A	nil	nil	nil	A C P	No growth	Pink, liquefying	nil	A
43 <i>B. buccalis maximus</i>	Growth	A	nil	nil	nil	A and C	No growth	White, slow liquefaction	nil	A
44 <i>B. mesentericus</i> <i>vulgatus</i> (K)	Growth	D	A, very slight	A, very slight	D	slight A C and P	No growth	Rapid liquefaction	nil	A
45 <i>B. mesentericus</i> <i>fuscus</i> (K)	Growth	D	nil	slight	D	slight A C and P	No growth	Slow liquefaction	nil	A
46 <i>B. mesentericus</i> <i>niger</i> (K)	Growth	D	nil	A, very slight	D	slight A C and P	No growth	Coarse feathery out- growths, liquefying	nil	A
47 <i>B. pyocyaneus</i>	Growth	nil	nil	nil	nil	A C P	Flat, transparent, pinkish brown. No haze	Pink, rapid liquefaction, complete colouration of serum	nil	Green
48 <i>B. Zopfii</i>	Growth	nil	nil	nil	D	nil	<i>Surface</i> : grey, translucent, with filmy, hairy edge. No haze. <i>Deep</i> : like balls of wool	White, feathery, non- liquefying	nil	A
49 <i>B. disciformans</i> (Zopf) 50 <i>B. brunneus</i>	Growth Growth	slight A nil	nil nil	nil nil	A nil	A C P Alkaline	No Growth	White, slow liquefaction	nil	A
51 <i>B. megatherium</i>	Growth	nil	nil	nil	nil	A and C P	Round, raised, opaque- greyish white. No haze	White, non-liquefying	nil	A
52 <i>V. Finkler-Prior</i>	Growth	nil	nil	nil	nil	slight A	No growth	Slight liquefaction	nil	A
53 <i>M. ureae</i> (K)	Growth	nil	nil	nil	A	A, very slight	No growth	Pink, liquefying	nil	nil
54 <i>Sarcina lutea</i>	Growth	nil	nil	nil	nil	nil	No growth	White, non-liquefying	nil	nil
								Yellow, liquefying	nil	A, very slight

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization. (K) Obtained from Král's laboratory.

DIVISION IV

Organism	Tauro- cholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum	GLYCERINE	
									48 hrs.	6 days
55 <i>B. anthracis</i>	no growth	A	nil	nil	A	nil	No growth	Greyish, non-liquefying	nil	A
56 <i>B. anthracoides</i> (K)	no growth	D	nil	D	slight A	A C P	No growth	Abundant, white, non-liquefying	nil	slight A
57 <i>B. diptheriae</i>	no growth	nil	nil	nil	nil	nil	No growth	White, non-liquefying	nil	nil
58 <i>B. faecalis alkaligenes</i> (K)	no growth	nil	nil	nil	nil	nil	No growth	Slow growth, non-liquefying	nil	nil
59 <i>B. fluorescens albus</i> (K)	no growth	D	nil	A, very slight	D	slight A	No growth	No growth	nil	A
60 <i>B. fluorescens putidus</i> (K)	no growth	nil	nil	nil	nil	nil	No growth	White, slow-liquefaction	nil	nil
61 <i>B. necrodentalis</i> [(K)]	no growth	nil	nil	nil	nil	A	No growth	No growth	nil	nil
62 <i>B. Stutzeri</i> (K)	no growth	nil	nil	nil	nil	A	No growth	Pinkish, non-liquefying	nil	nil
63 <i>B. subtilis</i>	no growth	A	nil	nil	A	A C P	No growth	Pink, liquefying	nil	A
64 <i>B. xerosis</i>	no growth	nil	nil	nil	nil	nil	No growth
65 <i>M. tetragenus</i>	no growth	nil	nil	nil	nil	nil	No growth	Opaque-white, non-liquefying	nil	nil
66 <i>Sarcina aurantiaca</i> (Flügge) (K)	no growth	nil	nil	nil	nil	nil	No growth	Orange, non-liquefying (later liquefaction)	nil	nil
67 <i>Sarcina flava</i> (De Bary) (K)	no growth	nil	nil	nil	nil	nil	No growth	Yellow, liquefying	nil	nil
68 <i>Sarcina ventriculi</i> (K)	no growth	nil	nil	nil	nil	nil	No growth	Brown, non-liquefying (later liquefaction)	nil	A, very slight
69 <i>Saccharomyces ellipsoideus</i>	no growth	nil	nil	nil	A	nil	No growth	Scanty, white, non-liquefying	nil	nil
70 <i>Cladothrix dichotoma</i>	no growth	nil	nil	nil	nil	nil	No growth	Brown, liquefying	nil	nil
71 <i>Torula alba</i>	no growth	nil	nil	nil	nil	nil	No growth
72 <i>Torula rubra</i>	no growth	nil	nil	nil	nil	A	No growth	No growth
73 <i>Streptococcus pyogenes</i>	no growth	...	A	A, very slight	No growth

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization. (K) Obtained from Král's laboratory.

II

In the first part of this paper we have shown how it is possible to group the chief micro-organisms with regard to their behaviour and growth in Taurocholate-glucose broth.

In order to further separate and distinguish the various members of each group, we have studied their fermentative power upon the following sugars, viz., glucose, lactose, mannite, and cane, upon glycerine, their reaction in litmus milk, and their growth on blood serum and Taurocholate-lactose agar. The results are shown below in tabular form.

TEMPERATURE AND LENGTH OF TIME OF INCUBATION

In this table in every instance the temperature of incubation has been 42° C. The duration of incubation has been forty-eight hours, except where otherwise stated.

NATURE OF MEDIA EMPLOYED

The composition of the various media employed is as follows:—

Litmus taurocholate glucose broth: Sod. taurocholate, 0.5 per cent.; glucose, 0.5 per cent.; peptone, 2.0 per cent.; water, 100 cc.

Litmus glucose broth: Glucose, 0.5 per cent.; peptone, 2.0 per cent.; water, 100 cc.

Litmus lactose broth: Lactose, 1.0 per cent.; peptone, 2.0 per cent.; water, 100 cc.

Litmus mannite broth: Mannite, 1.0 per cent.; peptone, 2.0 per cent.; water, 100 cc.

Litmus cane sugar broth: Cane sugar, 1.0 per cent.; peptone, 2.0 per cent.; water, 100 cc.

Litmus glycerine broth: Glycerine, 1.0 per cent.; peptone, 2.0 per cent.; water, 100 cc.

Taurocholate lactose agar: Agar, 1.5 per cent.; sod. taurocholate, 0.5 per cent.; lactose, 1.0 per cent.; peptone, 2.0 per cent.; water, 100 cc.

An examination of the table of reactions of Division I leads to the conclusion that certain organisms, which have been described as distinct from each other, are in reality very closely allied, or identical. Thus, Nos. 1, 2, and 3 are practically alike. It has been said that No. 2, *B. acidi lactici* (HUEPPE), is stained by GRAM's method. It is true that a faint coloration may be observed after this method of staining, but we consider that this amount of colour does not warrant the statement that it 'stains by GRAM.' We think that the result of GRAM's method should be considered positive only when the organism is of the well known blue-black colour.

Even though Nos. 4, 5, 6, and 7 differ from the preceding in their fermentative action on cane sugar, we do not think that this justifies separation into a special group. No. 15 must also be put in the same category.

No. 16 seems to be closely allied to the above organisms, but slowly liquefies gelatine.

The same remarks apply to No. 17. Throughout the whole of our numerous experiments we have never yet met with this micro-coccus; therefore, though it is said to occur in the air, it is improbable that it will invalidate our proposed test for faecal contamination. As regards No. 14, the culture with which we have experimented differs from the usual description of this organism, in that it does not ferment lactose or glycerine. The usual description would lead to its inclusion in the coli group.

The remaining organisms, Nos. 8 to 13, may be classed together, as forming the 'GAERTNER' or 'para-colon' group.

Passing on to Division II, No. 18, *B. typhi abdominalis* appears to stand out by itself.

No. 19, *B. pyogenes foetidus*, though incapable of fermenting sugars, resembles so closely the B.C.C. group in the appearance of its colonies on taurocholate lactose agar, that we are inclined to consider it an attenuated member of that group; more especially as we have isolated organisms which have lost their power of gas production after prolonged cultivation.

It is peculiar that though Nos. 25 to 30 resemble each other in almost every respect, we were unable to obtain any growth upon taurocholate lactose agar plates from Nos. 28, 29, and 30.

The colonies of No. 31, *B. rhinoscleroma*, and No. 48, *B. Zopfi*, upon taurocholate lactose agar, are indistinguishable from each other.

The remaining organisms call for no further remark.

Now it is obvious, from a consideration of the tables given above, that of all the organisms with which we have worked there are only seventeen which give the complete reaction in taurocholate broth; and of these thirteen are distinctly intestinal.

With regard to the remaining four, we consider:—

1. That as milk is so exposed to contamination, and the *B. acidi lactici* (HUEPPE) bears such a close relationship to *B. coli*, it should be placed in the same group.

2. That the *B. pneumoniae* (FRIEDLÄNDER) will not invalidate the test, because it is a noxious organism, and in the present state of our knowledge can not be said to be non-intestinal.

3. That the *B. cloacae* (JORDAN), having apparently been isolated only from waters certainly contaminated, may be looked upon as evidence of pollution.

4. That the *M. candicans* (FLÜGGE) may be neglected, on account of the rarity of its appearance in our work.

It is possible that future research may reveal sources of error or confusion, but up to the present this test has in our hands proved itself simple and reliable.

MILK AS A VEHICLE OF TUBERCLE

MILK AS A VEHICLE OF TUBERCLE AND PRESENT LOCAL LEGISLATION IN REGARD TO IT

BY E. W. HOPE, M.D., MEDICAL OFFICER FOR THE CITY OF LIVERPOOL

It is obvious that numerous points are involved in the subject, some of which are difficult to dissociate from questions other than those which concern tuberculosis only. For example, measures taken with the sole object of checking an even more destructive form of disease, viz., diarrhoea, have proved incidentally a safeguard against tuberculosis, whilst, on the other hand, measures directed against tuberculosis have afforded a valuable protection from other forms of disease.

Sterilization of milk possesses one conspicuous advantage, viz., that the application of the safeguard is within the reach of every reasonably prudent and careful household, consequently for ease of application it is beyond any comparison with other preventive measures. The objections to it do not appear to be important, but there are the facts to be reckoned with, that in the lower quarters of every great town there are thousands of families neither prudent nor careful, and also that the population of this country as a rule prefer to take their milk raw. This preference results no doubt partly from thoughtlessness and partly from habit. Young children are trained to take it raw, and the belief is widespread, that if the milk is raised in temperature to say 200° F., or even still nearer the boiling point, it is altered in flavour and constitution, and is of less nutritive and digestive value than when it is given raw; the raw milk in fact is regarded as more nearly approaching the natural milk of the mother.

There is no clinical evidence whatever to show that sterilized or even boiled milk is less nutritious and valuable than raw milk. On the other hand, raw cows' milk, in addition to the risk of tuberculosis, brings many others. The process of milking may involve dirt from a dirty milker, from dirty udders into a dirty milk pail. From this it may be passed through a dirty strainer into a dirty railway can. It is discharged from the railway can into smaller vessels in which it is hawked about the dusty streets, passing through some half-dozen other pots and pans before it reaches the nursery or the table of the consumer, involving a host of possible sources of contamination, not excepting the contamination of Tubercle Bacillus, in fact, it may be safely said, there is no article of food in common use so constantly exposed to contamination, or so susceptible of contamination, as raw milk. The milk, on the

other hand, as Nature intended it to be given, is never once exposed to air, passing directly and at the time of its manufacture in the gland, to the stomach of the young animal, and, apart from the possibility of disease in the gland, is bacteriologically clean and pure.

Sterilization, valuable as it is as a final safeguard against tuberculosis, is after all only an expedient, and must not be put into so much prominence that the importance of the safeguard afforded by keeping the cows healthy is lost sight of, although we cannot take it for granted, in considering the merits of different methods, that essential accessories common to them all will be observed. The one merit of sterilization is that it is an expedient easy of application and presenting few administrative difficulties. Beyond any question the ultimate advantage lies in obtaining the milk from herds free from tuberculosis. It is, in fact, comparable with the advantage of obtaining drinking water from a pure source, instead of taking it from a contaminated one and relying upon purification afterwards. The first aim must be to ensure that the source of the milk is pure; in other words, that the cows are free from tuberculosis, or if this, under existing conditions of the law and public opinion, is unattainable, that they shall at least be free from any tuberculous disease of the udder, or any tumour or condition of the udder simulating tuberculous disease, or, having regard to difficulties in diagnosis, we may with advantage go even a step further, and demand that the udder in all cows from which milk is taken for human food shall be in a perfectly normal condition.

The main causes of tuberculosis in cows are notorious: close confinement in ill-ventilated, badly-lighted, ill-constructed and dirty cowsheds—defects all as easy to remedy as is removal from the cowshed of the obviously tuberculous animal before it can cause infection of the rest.

In the city of Liverpool about 26,000 gallons of milk are consumed every day; one-half of it comes from cows, about 6,000 in number, kept within the city, the other half comes from cows kept in the country, and is sent in by rail. Within recent years that part of the milk supply which comes from cows kept within the city has been practically free from tuberculosis. This has been brought about by the sanitation of the cowsheds, adequacy of air, light, and cleanliness, by systematic and frequent inspection of the cows by qualified inspectors with veterinary help, by frequent bacteriological analysis of the samples of milk: these are the measures which have effected this end. I do not say that out of the 6,000 cows in the city there is not a single one affected with tubercle, but merely that there are few with such forms of tuberculous disease as would be likely to contaminate the milk supply.

These methods and this system of inspection were not initiated without difficulty and opposition. There is no opposition now; every person acquiesces in advantages which have been gained. But there is another aspect to the question. Only one half of the quantity of milk consumed in Liverpool is supplied from the

city, the remaining half comes from the country districts, but, it may be said, if the cows kept in the cowsheds within a great and populous city are healthy, those coming from the sunny meadows of the country, with their fertile pastures and ample land, are free also. Unfortunately, experience does not bear this out, the milk sent in from the country is more frequently tuberculous; thus out of 422 town samples examined during 1899 and 1900, five were tubercular, being a little more than one per cent., but out of 490 country samples taken during the same period, twenty were tubercular, being a trifle over four per cent. How can we protect ourselves against this? A special Act of Parliament applying to a few great towns, including Liverpool, gives special powers to exclude from the city, under a penalty, the milk coming from the country cowsheds in which tuberculous cows are kept under dirty and insanitary conditions. But if it is difficult to deal with and supervise the supply within our own city, it is evidently both costly and difficult to maintain a staff to send, under the special Act of Parliament, to the insanitary and tubercle-ridden cowsheds of the country cowman; but having overcome these difficulties, the broad national question comes in, for, although we succeed in protecting ourselves, what happens with regard to the diseased cows and the diseased milk? The dealer refrains from sending diseased milk to the protected city, but what is there to prevent him from sending his milk for sale and consumption to a district where no special Act of Parliament exists to enable the community to protect itself, or from selling his diseased cows to a dairyman in another locality. This is not the way to secure a supply of milk from herds free from tuberculosis, but there can be little doubt that the action of the great cities will not only protect themselves, but will, to a certain extent, protect the country districts also, and will strengthen the hands of rural sanitary authorities. No doubt the great cities are financially better able to protect themselves; they have their larger and more costly staff, they have their bacteriological laboratories, their veterinary and medical officers, but at best they are but valuable allies to the rural sanitary authorities, and these, after all, must take their own action, since the protection the cities afford them is an indirect vicarious one, and as in cases already alluded to, there is nothing to prevent the cow-keeper from sending his diseased produce to rural districts, after he has been prohibited from sending it to the great cities. Furthermore, the undoubted decline in the proportion of tuberculous milk sent in from the country may really mean that a larger proportion is consumed elsewhere. The subject is quite important enough for a Government Department, *e.g.*, the Local Government Board to take in hand and appoint a special staff to supervise the milk supply and all appertaining to it throughout the country.

It is quite possible to ensure that the milk supply shall come from cows free from tuberculosis. Difficulties, from ignorance, obstruction; and active opposition may be taken for granted, but these must be overcome, and the cow-keeper will

learn in time that his own interests are identical with those of his customers, and by keeping healthy cows in a sanitary condition he will be a gainer in every way.

It is only right to emphasize the fact that during the last year the samples of milk taken at the railway stations on arrival from the country did not appear to be more frequently tubercular than the samples taken from the town. This may indicate one of two things; either a general improvement in the country cowsheds under the stimulus of city action, or as in more cases than one which have come under notice, dairymen, who have been detected in sending in tuberculous milk, have refrained altogether from sending milk to Liverpool, and now send their milk elsewhere. These are points not to be lost sight of.

The Liverpool Corporation Act, 1900, contained, amongst others, the following important clauses, designed to protect consumers of milk from the dangers of tuberculosis:—

17. In this Part of this Act—

‘Dairy’ shall include any farm, farmhouse, cowshed, milk store, milk shop, or other place from which milk is supplied, or in which milk is kept for purposes of sale:

‘Dairyman’ shall include any cowkeeper, purveyor of milk, or occupier of a dairy:

‘Medical Officer’ means the medical officer of health for the city, and includes any person duly authorized to act temporarily as medical officer of health.

18. Every person who knowingly sells or suffers to be sold or used for human consumption within the city the milk of any cow which is suffering from tuberculosis of the udder, shall be liable to a penalty not exceeding ten pounds.

19. Any person the milk of the cows in whose dairy is sold or suffered to be sold or used for human consumption within the city, who after becoming aware that any cow in his dairy is suffering from tuberculosis of the udder, keeps or permits to be kept such cow in any field, shed, or other premises along with other cows in milk, shall be liable to a penalty not exceeding five pounds.

20. Every dairyman who supplies milk within the city, and has in his dairy any cow affected with, or suspected of, or exhibiting signs of tuberculosis of the udder, shall forthwith give written notice of the fact to the medical officer, stating his name and address, and the situation of the dairy or premises where the cow is.

Any dairyman failing to give such notice shall be liable to a penalty not exceeding forty shillings.

21. (1) It shall be lawful for the medical officer or any person provided with and, if required, exhibiting the authority in writing of such medical officer, to take within the city for examination samples of milk produced, or sold, or intended for sale within the city.

(2) The like powers in all respects may be exercised outside the city by the medical officer or such authorized person if he shall first have obtained from a justice, having jurisdiction in the place where the sample is to be taken, an order authorizing the taking of samples of the milk, which order any such justice is hereby empowered to make.

22. (1) If milk from a dairy situate within the city is being sold or suffered to be sold or used within the city, the medical officer or any person provided with and, if required, exhibiting the authority in writing of the medical officer, may, if accompanied by a properly qualified veterinary surgeon, at all reasonable hours, enter the dairy and inspect the cows kept therein; and if the medical officer or such person has reason to suspect that any cow in the dairy is suffering from tuberculosis of the udder he may require the cow to be milked in his presence and may take samples of the milk, and the milk from any particular teat shall, if he so requires, be kept separate and separate samples thereof be furnished.

(2) If the medical officer is of opinion that tuberculosis is caused or is likely to be caused to persons residing in the city from consumption of the milk supplied from a dairy situate within the city or from any cow kept therein he shall report thereon to the Corporation, and his report shall be accompanied by any report furnished to him by the veterinary surgeon, and the Corporation may thereupon serve upon the dairyman notice to appear before them within such time, not less than twenty-four hours, as may be specified in the notice to show cause why an order should not be made requiring him not to supply any milk from such dairy within the city until the order has been withdrawn by the Corporation.

(3) If the medical officer has reason to believe that milk from any dairy situate outside the city, from which milk is being sold or suffered to be sold or used within the city, is likely to cause tuberculosis in persons residing within the city, the powers conferred by this section may in all respects be exercised in the case of such dairy, provided that the medical officer or other authorized person shall first have obtained from a justice having jurisdiction in the place where the dairy is situate an order authorizing such entry and inspection, which order any such justice is hereby empowered to make.

(4) Every dairyman and the persons in his employment shall render such reasonable assistance to the medical officer or such authorized person or veterinary surgeon, as aforesaid, as may be required by such medical officer, person, or veterinary surgeon for all or any of the purposes of this section, and any person refusing such assistance or obstructing such medical officer, person, or veterinary surgeon in carrying out the purposes of this section shall be liable to a penalty not exceeding five pounds.

(5) If in their opinion the dairyman fails to show cause why such an order may not be made as aforesaid the Corporation may make the said order and shall forthwith serve notice of the facts on the county council of any administrative

county in which the dairy is situate and on the Local Government Board, and, if the dairy is situate outside the city on the council or borough or county district in which it is situate.

(6) The said order shall be forthwith withdrawn on the Corporation or their medical officer being satisfied that the milk supply has been changed or that it is not likely to cause tuberculosis to persons residing in the city.

(7) If any person after any such order has been made supplies any milk within the city in contravention of the order or sells it for consumption therein he shall be liable to a penalty not exceeding five pounds, and if the offence continues, to a further penalty not exceeding forty shillings for every day during which the offence continues.

(8) A dairyman shall not be liable to an action for breach of contract if the breach be due to an order under this section.

(9) The dairyman may appeal against an order of the Corporation under this section or the refusal of the Corporation to withdraw any such order either to a petty sessional court having jurisdiction within the city or at his option, if the dairy is situate outside the city, to the Board of Agriculture who shall appoint an officer to hear such appeal. Such officer shall fix a time and place of hearing within the city and give notice thereof to the dairyman and the town clerk not less than forty-eight hours before the hearing. Such officer shall for the purposes of the appeal have all the powers of a petty sessional court.

(10) The Board of Agriculture may at any stage require payment to them by the dairyman of such sum as they deem right to secure the payment of any costs incurred by the Board of Agriculture in the matter of the appeal.

24. Offences under this Part of this Act may be prosecuted and penalties may be recovered by the Corporation before a petty sessional court having jurisdiction in the place where the dairy is situate or the offence is committed and not otherwise.

THE EXCRETORY AND TUBERCULAR
CONTAMINATION OF MILK

THE EXCRETORY AND TUBERCULAR CONTAMINATION OF MILK

By RUBERT BOYCE

As the result of analysis which have been made in the Laboratory during the last eighteen months, namely of 1,020 samples of milk, and 733 samples of water, it will be seen at a glance, that, as compared with water, milk is a highly contaminated substance.

The average number of bacteria in the water in Liverpool is 28 in 733 samples. The *B. coli* was found only seven times, and a gas forming anaerobe* in none of the samples. This water from its source to its supply to the consumer is under the strictest possible control. Inspectors are on the watershed, and are stationed along the pipe line; the filtration beds and reservoirs are under strict supervision and bacteriological investigations are made daily. The result is a pure supply, and no fear of infection by means of it.

The contrast between milk and water is very remarkable, and unmistakably shows that the milk supply of the country constitutes a very strong element of danger. For example, leaving out the total number of bacteria per c.c. which is so great that it is not to be compared with water, it will be seen in the Tables appended that *B. coli* is present 205 times in 1,026 samples or 19·09 per cent., and that a gas forming anaerobe is present 113 times. This means that owing to the crude methods of collecting and dealing with milk that excretory contamination is liable to occur to the extent of 19·09 per cent. If water contained such an average percentage its use would be immediately forbidden. How this extraordinary degree of contamination can take place is only too well known to those who have entered into the question of milk production. The significance of the contamination is obvious. If *B. coli* is present, so may pathogenic organisms, such as the *B. typhosus*. The *B. coli* not only indicates dirt and intestinal contamination, but it also may become harmful in itself and give rise to intestinal inflammations. It shows that if such neglect of the rules of cleanliness can take place in the shippon, in all probability the health of the animal giving the milk is equally neglected. And what do we find? Of the 1,026 samples 27 are tubercular, giving a percentage of 2·6 per cent.

In the majority of these cases the udder was obviously diseased, and yet the milk of the animal was consumed. If neglect such as this can occur in the case of tuberculosis of the udder, it is quite certain that there are animals suffering from

* Obtained by heating the milk at 80°C for fifteen minutes, and incubating anaerobically. As in all cases an inoculation into the guinea pig has not been made we cannot say that the organism is the *B. enteritidis sporogenes*.

generalized tuberculosis and other diseases, the milk of which is sold to the public although it is dangerous to the health of man. This disregard of care would not be tolerated in any other article of consumption, as far as I am aware.

If intelligent supervision is rendering the water supply of large towns above suspicion, surely we have a right to demand, and to insist upon, similar care in the case of the milk supply. As it is well known, milk dealers have recognized the danger of raw milk, and have long since taken to condensing and sterilizing and adding preservatives. In certain cases this departure has been a great boon, but it has been abused; the condensed milk is not sterile, we do not know by its constituents whether bacterial products are there or not. Sterilized milk, unless the source is known, is no guarantee that the milk was pure to commence with. The addition of preservatives is in the vast majority of cases a cloak of the worst description, and ought to be as strenuously forbidden as it is in Germany. None of these methods strike at the root of the evil. Intelligent supervision at the place of production and during distribution is necessary. If the milk producers themselves do not brace themselves up and see that it is to their advantage to improve their supply and its keeping properties, municipal authorities must do so. In one or two large cities the municipality has undertaken the task in earnest and with most striking results. I quote from Liverpool: of 372 samples of milk taken from the Liverpool shippens, 7 were tubercular, 41 contained *B. coli*, and 27 a gas-forming anaerobe; of 414 railway-borne samples and therefore coming to Liverpool from a distance, 11 were tubercular, 105 contained *B. coli*, and 54 the gas-forming anaerobe. This shows that supervision in the case of the town shippens is beginning to have its effect, and that the milk has become purer than that from the country. The country-produced milk, with all the advantages which the country offers, is bad, for there is an almost total lack of organized supervision, and gallons of contaminated milk are allowed to find their way into vast populations to swell the chronic disease rate, the infant mortality, and the expenditure.

But why, finally, should this work altogether be left to Corporations. Cannot the milk producers in a county, for instance like Cheshire, establish a laboratory where everything relating to milk may be studied; where the best means of diagnosing tubercle may be investigated, where the purity of the milk may be checked, and where the best means of cleansing and sending out milk may be worked out. In Germany, the co-operation between the butchers and the authorities is very striking, and the butcher gains, for very little is wasted.

The following case which has recently come under my notice shows the intolerable ignorance which still exists as regards the treatment of milk. Samples of milk were taken separately from the cows in a shippon, but instead of the milker washing his hands between each milking, he spits on them. Two of the samples of milk are returned as tubercular, by the guinea pig inoculation test, but subsequently

only one cow is proved to be suffering from tubercle. But it is obvious that the milker with his hands contaminated with the bacilli from the diseased cow may have infected the outside of the teats of any of the other cows, and so have inoculated the second sample. It is probable that this may be one of the ways in which primary tuberculosis of the udder is produced. Again, the milker might have been tubercular himself, and might have infected the cow by this most objectionable habit, which, I trust, is exceptionable. This points to one other conclusion, and that is, if a tubercular cow is found in a shippon, everything, udders, pails, walls, floors, should be thoroughly disinfected as in the case of anthrax. We run more danger from tubercle than anthrax and proper precautions should be taken. One tubercular cow may infect a whole shippon, and therefore the milk supply from it.

TABLES SHEWING THE COMPARATIVE FREQUENCY OF CONTAMINATION IN MILK,
WATER, AND SHELL FISH

MILKS

Source				Number of samples examined	Bacillus coli communis	Gas forming anaerobe	Number of tubercular milks
Town	372	41	27	7
Railway	414	105	54	11
'B'	105	32	3	5
'S'	4	2
'W'	125	22	28	4
'W'	6	3	1	...
Totals				1,026	205	113	27

WATER

Number of samples examined	Number of bacteria per cc.	Bacillus coli communis	Gas forming anaerobe
733	28	7	0

SHELL FISH

Number of samples examined	Bacillus coli communis	Gas forming anaerobe
217	42	50

REPORT TO THE MEDICAL OFFICER OF THE
BACTERIOLOGICAL EXAMINATIONS MADE
FOR THE CITY OF LIVERPOOL DURING
THE YEAR 1900

REPORT TO THE MEDICAL OFFICER OF THE BACTERIOLOGICAL EXAMINATIONS MADE FOR THE CITY OF LIVERPOOL DURING THE YEAR 1900

BY RUBERT BOYCE

BACTERIOLOGICAL EXAMINATIONS AND ANALYSES

The work has comprised :—

- (a) Examination of food stuffs of various kinds.
- (b) Regular examination of water supplied to the City.
- (c) Examinations into suspected cases of rabies, anthrax, glanders, etc.
- (d) Examination for diagnostic purposes in suspected cases of diphtheria, typhoid fever, tubercular sputum, etc.
- (e) Special investigations.

Every food-stuff and every sample of water is analysed for the presence of

(1) *Bacillus coli* ; (2) *Bacillus enteritidis sporogenes*.

Every sample of milk, cream, butter, margarine, and cheese, is, in addition, examined for the presence of the *Bacillus tuberculosis* by inoculation.

In every sample of water the number of bacteria present in the cubic centimetre is also noted.

To facilitate these operations special apparatus has been constructed in the laboratory, and many of the operations have been simplified by their use.

With regard to (a) the total number of samples of food-stuffs taken for bacteriological examinations during the year 1900 were as follows :—

1,067 Foods
101 Samples of Water
39 Miscellaneous examinations

In addition a very large number of bacteriological examinations were made of suspected Tubercular, Typhoid, and Diphtheria cases for the medical practitioners of the district.

Five hundred and fifty-six Typhoid and Diphtheria examinations.

The following is a list of food-stuffs examined:—

Sample	Number	Sample	Number
Bloaters (Tinned)	1	Oleo	1
Bloater Paste	9	Orange Butter	2
Bovril	1	Oysters (Tinned)	2
Brawn	3	Periwinkles	22
Bottled Plums	2	Polony	3
Boiled Rabbit (Tinned)	1	Preserved Tomatoes	11
Bottled Gooseberries	1	„ Pineapple	6
Black Treacle	1	„ Peaches	1
Butter	21	„ Apricots	2
Cockles	35	„ Peas	5
Cream	8	Picalilli	1
Condensed Milk	25	Pineapple Butter	1
Cheese	13	Pudding	1
Crab Paste	1	Potted Shrimps	3
Chutney	4	„ Beef	2
Chicken, Ham, and Tongue	2	„ Lobster	2
Chicken and Ham	4	Pork, Boiled and Smoked	1
Cream Cheese	1	Pork Pie	3
Dripping	4	Potted Tongue (Tinned)	2
Extract of Coffee	4	„ Beef (Tinned)	7
Flour	6	„ Ham	6
Fresh Herrings	1	„ Mixed Game	1
Fluid Beef	3	Sauces	21
Fruit Cream	1	Sausages	16
Fruit Syrups	2	Sago	1
Food Jelly	1	Sardines (Tinned)	20
Golden Syrup	1	Sterilized Milks	9
Honey	4	Salmon (Tinned)	13
Herrings and Tomato Sauce	1	Sausages (Tinned)	1
Infants' Food	1	Sweetmeats	2
Jams	18	Sugar	1
Jellies	14	Suet	1
Lobster (Tinned)	4	Tapioca	1
Lard	6	Turkey and Tongue	3
Lemon Curd	7	Veal and Ham	2
Lemon Cheese	1		
Margarine	14		1,067
Mussels	32	Water	101
Margarine (Tinned)	1	Typhoid and Diphtheria	556
Mince Meat	2	Miscellaneous examinations	39
Milk	560		
Oysters	65		
Oatmeal	5	Total	1,763

MILK ANALYSES FOR THE YEAR

The total number of milks examined was five hundred and sixty. These were examined for the presence of:—

1. The *Bacillus tuberculosis*
2. The *Bacillus coli*
3. The *Bacillus enteritidis sporogenes*
4. Other bacteria

The *Bacillus tuberculosis* indicates that the animal from which the milk was taken was tubercular, or that the pails into which the milk was received, or the hands of the milker, were infected from previous contact with a diseased cow.

The *Bacillus coli* indicates contamination with dirt, of an intestinal origin, or possibly that the cow was suffering from inflammation of the udder.

The *Bacillus enteritidis sporogenes* indicates dust or intestinal contamination.

PRESENCE OF THE TUBERCLE BACILLUS

Of the five hundred and sixty samples examined for tubercle, one hundred and five guinea pigs died before the tubercular test was completed, leaving four hundred and fifty-five samples for the completion of the investigation. *Of this number nine proved tubercular*, five were found in *railway* borne milks, and four in *town milks*.

The greater frequency of tubercle in railway-borne milks was also noted last year. It is a very serious matter that tubercle is still so wide-spread in milk. When it is remembered that one tubercular cow may be the means of infecting the milking utensils, the hands of the milker, and even the teats of the other healthy animals, regulations to deal with infected animals cannot be too stringent.

PRESENCE OF THE *BACILLUS ENTERITIDIS SPOROGENES* AND THE *BACILLUS COLI*

The *Bacillus enteritidis sporogenes* was found twenty-six times in two hundred and fifty-five town samples of milk, and forty-two times in three hundred and five railway-borne samples.

The *Bacillus coli* was present fifteen times in the town milks, and forty times in the railway milks.

This is an exceedingly interesting and important result, for it shows that less care is taken in handling the country milk, and, therefore, that contamination much more frequently occurs. *Bacillus enteritidis sporogenes* appears most common in March and April; *Bacillus coli* in November and December.

In the case of the railway-borne milk, the *Bacillus coli* was most abundant in December, and this may indicate that, in addition to dirt contamination, a possible other source of the coli was inflammation of the udder.

With regard to the relationship of the *Bacillus coli* to the *Bacillus enteritidis* sporogenes, it has been found that very frequently the two organisms do not occur together. The significance of this is important as throwing light upon the significance of the *Bacillus enteritidis* sporogenes as an index of pollution. Where the *Bacillus coli* and *Bacillus enteritidis* sporogenes occur together this would be strong evidence that the *Bacillus enteritidis* sporogenes was of recent intestinal origin. But in a very large number of cases the *Bacillus enteritidis* sporogenes occurs alone. In these cases it is very hard to say what importance is to be attached to its presence, and unless an inoculation test of the virulence of the *Bacillus* is made, it would be impossible to say whether the *Bacillus* is *enteritidis* sporogenes or *butyricus*.

When dealing with a very large number of food-stuffs, it very greatly increases the work if the pathogenicity of the *Bacillus* which is isolated has to be tested each time.

Table showing the frequency with which the *Bacillus coli* and *Bacillus enteritidis* sporogenes occur alone and together in five hundred and sixty samples of milk analyzed.

Date			Number of Samples	<i>Bacillus coli</i> alone	<i>Bacillus enteritidis</i> alone	Together
January	45	5	1	...
February	45	1	3	1
March	55	2	11	3
April	41	3	13	3
May	50	4	9	3
June	48	...	3	2
July	44	1	6	...
August	45	1	5	...
September	44	...	4	...
October	57	4	2	2
November	40	11	2	...
December	46	15

TABLE SHOWING THE TOTAL NUMBER OF MILKS WHICH WERE EXAMINED DURING 1900 FOR
TUBERCLE, *BACILLUS COLI COMMUNIS*, AND *BACILLUS ENTERITIDIS SPOROGENES*

Month	Town	Bac. Coli Com.	Bac. Ent. Spor.	Rail.	Bac. Coli Com.	Bac. Ent. Spor.	Hospital	Bac. Coli Com.	Bac. Ent. Spor.	Total Number	Number of Tubercular Milks
January	...	21	3	...	20	2	...	4	...	45	1 Town
February	...	21	...	1	20	2	3	4	...	45	...
March	...	28	3	7	20	2	6	7	...	55	1 Railway
April	...	17	1	8	19	3	8	5	...	41	1 Town
May	...	20	5	4	26	2	8	4	...	50	1 Town
June	...	24	18	2	5	6	...	48	1 Town
July	...	20	1	1	20	...	4	4	1	44	...
August	...	20	...	2	20	1	1	5	...	45	2 Railway
September	...	22	...	2	16	...	2	6	...	44	...
October	...	31	21	6	4	5	1	57	2 Railway
November	...	10	2	1	25	7	1	5	2	40	...
December	...	21	20	13	...	5	2	46	...

TABLE SHOWING THE TOTAL NUMBER OF MILKS EXAMINED BACTERIOLOGICALLY FOR
TUBERCLE BACILLI FROM AUGUST, 1896, TO 31ST DECEMBER, 1900

Year	Total Number of Samples taken	TOWN SAMPLES			COUNTRY SAMPLES		
		Number taken	Tubercular	Percentage Tubercular	Number taken	Tubercular	Percentage Tubercular
1896	119	83	4	4·8 per cent.	36	5	14·0 per cent.
1897	150	63	4	6·3 "	87	5	5·0 "
1898	112	84	7	8·3 "	28	5	17·9 "
	381	230	15	6·5 per cent.	151	15	10·0 per cent.
1899	352	167	1	0·6 per cent.	185	15	8·1 per cent.
1900	560	255	4	1·5 "	305	5	1·6 "
	912	422	5		490	20	
Totals ...	1,293	652	20	3·0 per cent.	641	35	5·3 per cent.

RESULTS OF ANALYSES OF BUTTER, CREAM, STERILIZED MILK, CONDENSED MILKS, CHEESE, LARD, AND MARGARINE

Butter. Twenty-one samples were analysed and the tubercle bacillus found in one case. If tubercle is present in milk, it can also be present in butter, cream, and margarine, and, therefore, the finding it in these food-stuffs is a further reason for increasing the vigilance of dairy supervision.

Creams. Eight samples of cream were examined and the *Bacillus coli* found twice and the *Bacillus enteritidis sporogenes* once.

Sterilized Milks. Of the nine samples examined one was found not to be sterile. The sterilization of milk is difficult on account of the presence of spore-bearing bacilli, the resistance of which to heat is very considerable.

Condensed Milks. Twenty-five samples were examined and the great majority were not sterile. There is no doubt that condensed milk is a most unsatisfactory product. Bacteria are usually present, and the milk, which was originally condensed, might have contained various products of the decomposition of bacteria. These products are masked subsequently by the large quantity of sugar present, but their irritant properties are not destroyed.

Cheese. Thirteen samples were examined. In one case the *Bacillus coli* was present, and in another sample the *Bacillus enteritidis sporogenes*. The probability is that in cheese, organisms like the *Bacillus coli* and *Bacillus tuberculosis*, which might have been originally present in the milk from which the cheese was made, tend to die out in the process of fermentation.

Lard and Margarine. Twenty-one samples were examined. No tubercle was found, and the *Bacillus enteritidis* in only one sample of margarine.

Bacteria present in Shell Fish. Some kinds of shell fish, like milk and milk products, are for the most part eaten uncooked; they are in consequence liable to convey infection if they become contaminated with pathogenic bacteria. Contamination may occur in the transit and storing of the shell fish, but more especially in the collecting grounds. It is not uncommon to find that sewage has access to oyster, mussel, and cockle beds. One hundred and fifty-four samples were examined for evidence of the *Bacillus coli* and *Bacillus enteritidis sporogenes*. The *Bacillus coli* was present seventeen times, the *Bacillus enteritidis* thirty-seven times. The *Bacillus coli* was more frequently present in oysters and mussels, the *Bacillus enteritidis* in periwinkles and cockles. Thus again, as in the case of the milks, there is little uniformity between the occurrence of these two bacilli. It is fortunate that *Bacillus coli* is not more abundant in shell fish in Liverpool, but no efforts must be spared to make the collecting grounds above suspicion of sewage contamination. In the case of cockles and mussels, this is difficult, as they are often taken from the mouths of estuaries where pollution unfortunately occurs to a great extent owing to the discharge of crude sewage.

Sausages. As in the case of sterilized milk, condensed milk, and raw foods generally, so in the case of sausages it is all important that the ingredients should be pure, otherwise the spice simply masks the bacterial changes, and does not destroy the ptomaines or indeed injurious bacteria. Seventeen samples were examined, and the *Bacillus coli* obtained in six samples and the *Bacillus enteritidis* in fourteen samples.

Tinned Meats, Fruits, and Vegetables. Ninety-four samples were examined, and in no case was either the *Bacillus coli* or *Bacillus enteritidis sporogenes* found. A few samples were not sterile.

Pastes and Potted Meats. In only one case out of eleven samples was the *Bacillus enteritidis sporogenes* found. Nine out of eleven were not sterile.

Cereals. Considerable interest attaches to the bacterial examination of these articles, because they are very liable to dust contamination. Thirteen samples were examined, of which four showed evidence of the *Bacillus enteritidis sporogenes*. No *coli* was found.

Jams. Jams have shown a freedom from dangerous or danger indicating bacteria. Many are sterile. Those which are not sterile only contain a few bacteria. There is no doubt that the greatest care must be used in the boiling and subsequent distribution of the jam into pots to ensure sterility and keeping properties.

The following is a summary of the chief investigations and analyses, together with references to the methods employed:—

I. THE INJURIOUS EFFECTS OF FOODS AND BEVERAGES PRESERVED WITH BORACIC AND SALICYLIC ACIDS

To test the injurious action of these preservatives, kittens, three weeks old, were fed with milk containing these preservatives in the proportion in which they were found in articles of diet. It will be seen from the table that the kittens fed on boracized milk from May 25 to June 2 failed not only to gain weight, but actually lost considerably in many cases. The control kittens, on the other hand, fattened in the usual manner. Further, the boracized kittens suffered in health, and were subject to diarrhoea. On June 8, a pure milk diet was substituted for the boracized milk, and the kittens rapidly gained in weight. These experiments confirm those which had been made in the previous year.

Further experiments made by Dr. GRÜNBAUM in this laboratory have shown that the addition of borax to milk to the extent of 0.4 per cent. by precipitating the calcium is sufficient to inhibit the action of the rennet ferment, whilst at the same time the inhibiting effect on the growth of pathogenic micro-organisms is practically *nil*. On the other hand, keeping milk cooled to 40 deg. F. almost entirely stops the growth of the bacteria.

Both the feeding and digesting experiments show that boracic acid in milk is injurious, and ought not to be added.

BORACIC ACID EXPERIMENTS

KITTENS FED WITH BORACIZED MILK CONTAINING ABOUT 82 GRAINS TO THE PINT					CONTROL KITTENS FED WITH PURE MILK				
Date 1900			Kitten	Weighed	Date 1900			Kitten	Weighed
May 26	No. 1	822 grms.	May 26	No. 1	550 grms.
"	" 2	602 "	"	" 2	595 "
"	" 3	715 "	"	" 3	710 "
"	" 4	765 "	"	" 4	530 "
"	" 5	620 "	"	" 5	570 "
May 30	No. 1	715 grms.	May 30	No. 1	624 grms.
"	" 2	602 "	"	" 2	616 "
"	" 3	702 "	"	" 3	818 "
"	" 4	751 "	"	" 4	624 "
"	" 5	540 "	"	" 5	632 "
June 2	No. 1	777 grms.	June 2	No. 1	670 grms.
"	" 2	580 "	"	" 2	648 "
"	" 3	717 "	"	" 3	850 "
"	" 4	755 "	"	" 4	680 "
"	" 5	500 "	"	" 5	643 "

ON JUNE 8, THE KITTENS WHICH HAD BEEN FED WITH BORACIZED MILK
WERE CHANGED TO A DIET OF PURE MILK

Date 1900	Kitten	Weighed	Date 1900	Kitten	Weighed
June 8	No 1.	880 grms.	June 8	No. 1	678 grms.
"	" 2	720 "	"	" 2	655 "
"	" 3	840 "	"	" 3	890 "
"	" 4	832 "	"	" 4	700 "
"	" 5	510 "	"	" 5	590 "
June 15	No 1.	800 grms.	June 15	No. 1	590 grms.
"	" 2	860 "	"	" 2	615 "
"	" 3	895 "	"	" 3	1,000 "
"	" 4	800 "	"	" 4	737 "
"	5 "	505 "	"	" 5	636 "
June 22	No 1.	Missing	June 22	No. 1	682 grms.
"	" 2	1,050 grms.	"	" 2	695 "
"	" 3	1,080 "	"	" 3	1,010 "
"	" 4	790 "	"	" 4	980 "
"	" 5	600 "	"	" 5	690 "

In the case of the salicylic acid experiments, one kitten fed on salicylized milk increased in weight from October 23, when the experiments were commenced, till December 4, when the experiments ended. A second kitten decreased in weight, and died on December 16. The third kitten at first increased in weight, and then began to lose, and died on November 27. Of the control kittens, the first and second increased in weight from the commencement to the end of the experiments; the third died too soon after the commencement of the observations for any deductions to be made. These experiments show that salicylic acid has an injurious effect, though less marked than boracic acid, but further research is necessary.

The injurious effects of formalin were fully dealt with in last year's Annual Report.

2. EXPERIMENTS AND OBSERVATIONS UPON THE SIGNIFICANCE OF THE BACILLUS ENTERITIDIS SPOROGENES

Like the *Bacillus coli*, this organism is systematically looked for in waters and food-stuffs. Dr. KLEIN has laid considerable stress upon its presence, as he considers that it is capable of causing diarrhoea much in the same way as the *Bacillus coli*.

During the year much evidence has been accumulated to show its distribution in food-stuffs, and special investigations have been made to determine its significance. Attention was especially drawn to this organism by a case of poisoning which was thought might be due to eating diseased salted fish. Examination of the dried fish showed the presence, amongst other bacteria, of the *Bacillus enteritidis sporogenes*. Subsequent examinations of numerous examples of dried fish, however, showed that this organism was normally present. A series of analyses of foods liable to dust contamination was then made, viz., wheat, barley, oats, oatmeal, flour, rice, cornflour, clovers, grasses, etc. Sixty samples were examined, and forty-one gave an enteritidis-like growth in milk, and thirty were fatal to guinea pigs when inoculated, and eleven produced an inflammatory reaction.

Further research demonstrated that the bacillus was widespread. The pathogenicity of the bacillus isolated was tested, in order to make certain that the bacillus isolated was that described by Dr. KLEIN.

The observations of the year's analyses show that the organism is abundant in milk and other food-stuffs, and our conclusions are that the *Bacillus enteritidis sporogenes* is much more widespread than the *Bacillus coli*, owing no doubt to its power of spore formation, and that, therefore, although originally derived from the intestine, its presence in a food is not of the same significance as that of the *Bacillus coli*. With regard to its pathogenicity in animals there is no doubt, but in man it is like the *Bacillus coli*, common to the intestine. It may be that certain forms of diarrhoea are due to an increased virulence of this organism in the intestine as in the case of diarrhoea associated with *Bacillus coli*, but further evidence of this is wanted.

3. EXPERIMENT TO DEMONSTRATE THE SIGNIFICANCE OF THE *BACILLUS COLI*

This organism is looked for in all samples of foodstuffs and water where bacteria are known or suspected to be present. The reason for this is that it is considered by many to be evidence of sewage contamination. In all the analyses it is therefore stated whether it is present or absent, and the result is that during the past twelve months a mass of evidence shows that the *Bacillus coli* indicates recent pollution or contact with inflammatory discharges.

Stream and rivulets, not obviously polluted, showed an absence of this organism in the quantities analysed. Sewage and sewage effluents, on the other hand, or streams near human habitations, showed the presence of the *Bacillus coli*. It has a very low degree of resistance, and soon perishes outside the alimentary tract. This was strikingly demonstrated in the roadways. If the season was dry and the roads dusty, the *Bacillus coli* was absent or very scanty in the dust. On the other hand, in the gutters along the side of the roads, which are usually moist and often receive garbage, the *Bacillus coli* were very numerous. Although the *Bacillus coli*

is normally found in the intestine of man and animals, and, therefore, cannot be said to be under these circumstances harmful, nevertheless cases do occur in which marked diarrhoea is found associated with great development of this organism in the intestine. Such cases of diarrhoea often occur in epidemic form, and the evidence is that under certain circumstances the *Bacillus coli* may become pathogenic, and produce inflammation in the alimentary tract.

Distribution of Tuberculosis. Dr. ELLIOTT conducted an interesting enquiry into the distribution of tuberculosis in Liverpool and the infectivity of houses in which patients have recently died. He examined the dust in four out of ten houses in which deaths had occurred seven to fourteen days previously, and found, by inoculation in the guinea pig, that the tubercle bacillus was present in one of them. In this infected house there had been carelessness in the disposal of the sputa, and cleanliness had not been observed during and after the patient's death. This is a very important observation, for it shows the danger of the consumptive's room not only during his illness but for some considerable time afterwards, and it also shows the value of the disinfection and cleansing carried out by the disinfecting staff when cases of phthisis are notified.

Plague investigations. During the year numerous rats and several suspected cases of Plague were examined by Dr. BALFOUR STEWART for the presence of the Plague bacillus, but none was found. To be ready in case of any emergency a stock of vaccine was prepared and kept in the laboratory, and, although no occasion arose in Liverpool for its use, it was supplied to other towns in England where cases had occurred. A large demand also arose for it owing to the outbreak in South Africa, and the total quantity supplied to municipalities, private individuals, the Colonial and War Offices, amounts to seventy thousand doses. At the present time a very large quantity is available for immediate use in case of any emergency. *Nature of the vaccine:* The vaccine is prepared after HAFFKINE's method, and consists of a sterilized broth cultivation of the virulent plague bacillus. It is put up in sterilized bottles containing a definite number of doses, and is most carefully sealed.

Investigation of 'Pink Eye' in Horses. A severe epidemic of this disease broke out during the year amongst the horses of the Corporation and in private stables.

Having failed to obtain evidence of an organism in the horses, numerous examinations were made of the discharges from the eyes and nose, and of all the organs of horses which were slaughtered whilst suffering from the disease. The organs were examined immediately after death, and included the nasal cavity, trachea conjunctiva, liver, spleen, kidneys, subcutaneous tissues, and heart. From the mucous membranes a characteristic bipolar *Bacillus* was isolated in large numbers in every case. The *Bacillus* was pathogenic to guinea pigs, producing fatal results or extensive oedema. This *Bacillus* was common to all the cases of Pink Eye; it was

abundant not only in the discharges but far back in the nasal cavity when the head of the horse was opened immediately after death, and it was pathogenic. From its cultural properties it appears to be a member of the *Bacillus coli* group. Without further observations it would be impossible to state whether this virulent coli form was the cause of the disease. The inquiry will be continued if another outbreak arises.

RABIES

Twenty dogs were examined for rabies, but fortunately in no case was rabies shown to be present.

Date	Result of Inoculation.
January 27	Not Rabies
February 28	do.
March 8	do.
March 9	do.
March 10	do.
April 7	do.
April 9	do.
April 20	do.
June 5	do.
June 11	do.
June 21	do.
July 5	do.
July 5	do.
July 15	do.
July 24	do.
July 31	do.
September 13	Rabbit died Sept. 21
September 21	Not Rabies
October 2	do.
November 6	Inoculation unsuccessful

BACTERIOLOGICAL ANALYSES OF CASES OF TYPHOID AND DIPHTHERIA IN THE CITY FEVER HOSPITALS

During the year the fever hospitals have availed themselves of the facilities of the Municipal Bacteriological Department, and five hundred and fifty-one specimens have been examined.

The following is a summary of the results :—Three hundred and thirty-six cases of diphtheria, two hundred and fifteen typhoid, four malaria, and one tuberculosis.

Of three hundred and thirty-six cases of diphtheria—

158 were positive

141 were negative

23 were no growth

14 were suspicious

336 total

Of two hundred and fifteen cases of typhoid—

124 gave a positive reaction

79 gave a negative reaction

7 were suspicious

5 were not examined for various reasons

215 total

No plasmodium malariae in any of the malaria specimens.

No tubercle bacilli were found in the specimen of sputum.

WATER ANALYSES

All the samples of water have been systematically examined for the presence of the *Bacillus coli* and the *Bacillus enteritidis sporogenes*, as well as for the total number of bacteria. The quantity of water used for each analysis has been one cubic centimetre.

The following are the sources which have been examined :—

Fortnightly Examinations—

Ashton Hall Tap.

Monthly Examinations—

PRESCOT—	{	Lake Vyrnwy Water
		Rivington Water
		The Mixed Water
WELLS—	{	Green Lane Well
		Windsor Well
		Dudlow Lane Well

The results show that the *Bacillus coli* has not been found present in any sample to the one cubic centimetre used.

The average number of Bacteria present in :—

1.	Ashton Hall Water	.	.	=19.9 per c.c.
2.	Vyrnwy Aqueduct	.	.	=19.6
3.	Rivington Aqueduct	.	.	=12.16
4.	Green Lane Wells	.	.	=81.0
5.	Windsor Well	.	.	=52.0
6.	Dudlow Lane Well	.	.	=63.9

ASHTON HALL—FORTNIGHTLY SAMPLES

Source	Date, 1900	Time of Collecting	Time of Investment	No. of Bacteria	B. Coli Comm.	B. Ent. Sporog.
Ashton Hall	Jan. 10	10-30 a.m.	10-37 a.m.	39	Absent	Absent
"	Jan. 23	2-30 p.m.	3-0 p.m.	16	"	"
"	Feb. 9	2-5 p.m.	4-30 p.m.	19	"	"
"	Feb. 20	2-15 p.m. 21st	10-30 a.m.	25	"	"
"	Mar. 10	9-30 a.m.	11-40 p.m.	6	"	"
"	Maa. 28	3-30 p.m.	5-0 p.m.	large No.	"	"
"	April 17	10-30 a.m.	11-0 p.m.	10	"	"
"	April 25	10-38 a.m.	11-40 a.m.	13	"	"
"	May 11	3-30 p.m.	5-0 p.m.	6	"	"
"	May 25	2-0 p.m.	3-0 p.m.	20	"	"
"	June 8	3-0 p.m.	4-0 p.m.	3	"	"
"	June 21	3-7 p.m.	4-0 p.m.	41	"	"
"	July 5	3-0 p.m.	3-30 p.m.	29	"	"
"	July 20	10-20 a.m.	11-30 p.m.	12	"	"
"	Aug. 4	9-40 a.m.	11-0 a.m.	30	"	"
"	Aug. 11	11-0 a.m.	12-15 p.m.	22	"	"
"	Sept. 12	11-10 a.m.	11-30 a.m.	5	"	"
"	Oct. 1	10-30 a.m.	11-0 a.m.	23	"	"
"	Oct. 9	10-30 a.m.	4-30 p.m.	10	"	"
"	Oct. 28	10-30 a.m.	11-0 a.m.	16	"	"
"	Nov. 5	10-30 a.m.	11-30 a.m.	73	"	"
"	Nov. 24	11-0 a.m.	11-40 a.m.	13	"	"
"	Dec. 7	4-10 p.m.	5-0 p.m.	9	"	"
"	Dec. 29	10-40 a.m.	11-30 a.m.	18	"	"

GREEN LANE WELLS—MONTHLY SAMPLES

G. Holt Well	Jan. 23	1-13 p.m.	3-0 p.m.	50	Absent	Absent
"	Feb. 20	12-55 p.m. 21st	10-30 a.m.	48	"	"
"	Mar. 27	9-15 a.m.	6-30 p.m.	26	"	"
"	April 28	2-25 p.m.	5-5 p.m.	17	"	"
"	May 25	11-25 a.m.	3-0 p.m.	6	"	"
"	June 22	2-15 p.m.	4-0 p.m.	11	"	"
"	July 20	9-5 a.m.	11-30 a.m.	48	"	"
"	Aug. 8	10-15 a.m.	3-50 p.m.	114	"	"
"	Sept. 20	9-8 a.m.	11-0 p.m.	32	"	"
"	Oct. 12	1-30 p.m.	4-30 p.m.	34	"	"
"	Nov. } Dec. }	Samples not taken, engines not working.				
J. Holmes Well	Jan. 23	1-15 p.m.	3-0 p.m.	Gelatine plate broken.		
"	Feb. 20	1-0 p.m. 21st	10-30 a.m.	440	Absent	Absent
"	Mar. 27	9-13 a.m.	6-30 p.m.	62	"	"
"	April 28	2-30 p.m.	5-5 p.m.	46	"	"
"	May	Samples not taken, engines not working.				
"	June 22	2-15 p.m.	4-0 p.m.	112	"	"

Source	Date	Time of Collecting	Time of Investment	No. of Bacteria	B. Coli Comm.	B. Ent. Sporog.
J. Holmes Well	July 20	9-5 a.m.	11-30 a.m.	40	Absent	Absent
"	Aug. 8	10-20 a.m.	3-50 p.m.	104	"	"
"	Sept. 20	9-11 a.m.	11-0 a.m.	240	"	"
"	Oct. 13	9-15 a.m.	11-30 p.m.	5	"	"
"	Nov. }	Samples not taken, engines not working.				
"	Dec. }					

DUDLOW LANE—MONTHLY SAMPLES

Dudlow Lane	Jan. 23	1-38 p.m.	3-0 p.m.	22	Absent	Absent
"	Feb. 20	1-20 p.m.	10-30 p.m.	240	"	"
"	Mar. 17	9-38 a.m.	6-30 p.m.	14	"	"
"	April 28	3-15 p.m.	5-5 p.m.	63	"	"
"	May 25	11-55 a.m.	3-0 p.m.	12	"	"
"	June 22	2-40 p.m.	4-0 p.m.	17	"	"
"	July 20	9-35 a.m.	11-30 a.m.	70	"	"
"	Aug. 8	9-15 a.m.	3-50 p.m.	20	"	"
"	Sept. 20	9-40 a.m.	11-0 a.m.	204	"	"
"	Oct. 12	2-15 p.m.	4-30 p.m.	10	"	"
"	Dec. 7	2-45 p.m.	5-0 p.m.	31	"	"

WINDSOR WELL—MONTHLY SAMPLES

Windsor Well	Jan. 23	2-10 p.m.	3-0 p.m.	16	Absent	Absent
"	Feb. 20	1-50 p.m.	10-30 a.m.	84	"	"
"	Mar. 27	10-0 a.m.	6-30 p.m.	15	"	"
"	April 28	3-55 p.m.	5-5 p.m.	43	"	"
"	May 25	12-15 p.m.	3-30 p.m.	44	"	"
"	June 22	3-0 p.m.	4-0 p.m.	64	"	"
"	July 20	9-55 a.m.	11-30 a.m.	75	"	"
"	Aug. 8	8-30 a.m.	3-50 p.m.	18	"	"
"	Sept. 20	10-15 a.m.	11-0 a.m.	126	"	"
"	Oct. 12	3-0 p.m.	4-30 a.m.	67	"	"
"	Nov. 8	1-40 p.m.	4-0 p.m.	38	"	"
"	Dec. 7	1-30 p.m.	5-0 p.m.	35	"	"

PRESCOT—MIXING WELL—MONTHLY SAMPLES

Mixing Well	Jan. 23	4-50 p.m.	6-45 p.m.	17	Absent	Absent
"	Feb. 20	5-53 p.m.	10-30 a.m.	—	"	"
"	Mar. 28	2-10 p.m.	5-0 p.m.	40	"	"
"	April 24	2-8 p.m.	25th 10-30 p.m.	12	"	"
"	May 23	2-15 p.m.	4-0 p.m.	13	"	"
"	June 19	1-58 p.m.	4-25 p.m.	224	"	"
"	July 17	2-25 p.m.	3-15 p.m.	48	"	"
"	Aug. 8	2-15 p.m.	3-50 p.m.	163	"	"
"	Sept. 12	2-15 p.m.	4-15 p.m.	4	"	"

REPORT OF BACTERIOLOGICAL EXAMINATIONS

199

Source	Date	Time of Collecting	Time of Investment	No. of Bacteria	B. Coli Comm.	B. Ent. Sporog.
Mixing Well	Oct. 9	2-10 p.m.	4-0 p.m.	37	Absent	Absent
"	Nov. 5	3-33 p.m.	5-20 p.m.	26	"	"
"	Dec. 4	2-7 p.m.	4-30 p.m.	29	"	"

PRESCOT—RIVINGTON WATER—MONTHLY SAMPLES

Rivington	Jan. 23	4-45 p.m.	6-45 p.m.	6	Absent	Absent
"	Feb. 20	3-50 p.m.	10-30 a.m.	5	"	"
"	Mar. 28	2-3 p.m.	5-0 p.m.	21	"	"
"	April 24	2-5 p.m.	10-50 p.m.	5	"	"
"	May 23	2-5 p.m.	4-0 p.m.	4	"	"
"	June 19	1-55 p.m.	4-25 p.m.	7	"	"
"	July 17	2-5 p.m.	3-15 p.m.	6	"	"
"	Aug. 8	2-5 p.m.	3-50 p.m.	8	"	"
"	Sept. 12	2-5 p.m.	4-0 p.m.	1	"	"
"	Oct. 9	2-5 p.m.	4-0 p.m.	27	"	"
"	Nov. 5	3-25 p.m.	5-20 p.m.	28	"	"
"	Dec. 4	2-5 p.m.	4-30 p.m.	28	"	"

PRESCOT—VYRNWY WATER—MONTHLY SAMPLES

Vyrnwy	Jan. 23	4-40 p.m.	6-45 p.m.	8	Absent	Absent
"	Feb. 20	3-45 p.m.	10-30 p.m.	4	"	"
"	Mar. 28	2-0 p.m.	5-0 p.m.	16	"	"
"	April 24	2-0 p.m.	10-30 a.m.	11	"	"
"	May 23	2-10 p.m.	4-0 p.m.	3	"	"
"	June 19	1-50 p.m.	4-25 p.m.	12	"	"
"	July 17	2-15 p.m.	3-15 p.m.	21	"	"
"	Aug. 8	2-10 p.m.	3-50 p.m.	73	"	"
"	Sept.	Sample not taken, reservoir being altered.				
"	Oct. 9	2-0 p.m.	4-0 p.m.	20	"	"
"	Nov. 5	3-30 p.m.	5-20 p.m.	16	"	"
"	Dec. 4	2-0 p.m.	4-30 p.m.	32	"	"

NOTE ON 'PINK-EYE' IN HORSES

NOTE ON 'PINK-EYE' IN HORSES

C. BALFOUR STEWART

AND

RUBERT BOYCE

During several weeks of the winter of 1900 an epidemic of 'Pink-eye' raged among the horses of Liverpool, assuming, at its height, so serious proportions that it was no unusual occurrence for 20 to 40 dead horses to have to be dealt with in one knacker's yard of the city.

The onset of the disease was very insidious, and the fatal termination often extremely rapid, for there were many cases in which the animals dropped down dead in harness. That the disease was of an infectious nature was evident from the fact of its running through a stable, and it was recognized as such in the treatment, for the affected animals were always isolated in those stables which were under proper veterinary charge.

Amongst those who had to do with horses there seemed to be a general opinion that the disease was the same as influenza in human beings, but, on enquiry, we were unable to meet with any instance of a case of influenza occurring from contact with sick horses, nor were we able to separate a microbe in any way similar to that of Pfeiffer.

An infectious disease of this nature, involving not only heavy loss on team owners but also considerable suffering to the animals, particularly those which were driven with the disease already on them, is one imperatively calling for investigation to discover the micro-organism concerned, and, if possible, to devise some means for conferring immunity.

Unfortunately for our investigation the epidemic came to an end somewhat suddenly, but we think it not uninteresting to put on record what few observations we made, with a view of prosecuting the enquiry further should an occasion again present itself.

Seven horses in all were examined, and the results of our observations are as follows :—

Horse 1. Suffering badly. A small quantity of blood was incubated in a sterile test tube, and another portion was inoculated on to the usual cultivation media, but no growth was obtained on any of the tubes, and the blood remained sterile.

Horse 2. Convalescent one week. The blood clotted very rapidly : it was likewise found to be sterile.

Horse 3. Convalescent. The blood also clotted rapidly, but did not form so large a buffy coat as in the previous case. It was sterile.

Horse 4. Suffering badly. The conjunctivae were greatly injected and a serous fluid was discharged from both eyes. The blood was tested and found to be sterile. A cover slip preparation was made from the eye discharge, it showed small bacilli and a tetra-coccus. Agar culture tubes and Petri dishes were inoculated with the eye discharge, and from this we obtained an almost pure culture of what, to save repetition, we called the characteristic bacillus, because of the uniformity with which it was met with in this and the following cases. It was a small diplococcus or diplo-bacillus which grew as a white opaque streak on gelatine, causing no liquefaction. On agar it grew more vigorously and slightly more opaque than *B. coli*. In glucose gelatine it formed gas. It showed motility in a hanging-drop preparation. The culture of one of the agar tubes was suspended in sterile water, and three guinea-pigs were inoculated with equal portions of the suspension. Two days afterwards one of the guinea-pigs had an oedematous swelling at the point of inoculation, it was killed, and there was found considerable subcutaneous oedema containing small diplococci. Agar tubes were inoculated from the oedematous fluid, and from the heart blood; those from the latter showed the characteristic bacillus both culturally and microscopically.

Horse 5. Suffering badly. Cultures from the conjunctiva showed the characteristic bacillus. A guinea-pig inoculated with some of the culture died in three days with an oedematous swelling similar to the last, and a cultivation from this gave the same bacillus.

Horse 6. Cultures were made from the lungs, trachea, nasal mucous membrane, conjunctivae, liver, spleen, kidney. The characteristic bacillus was recovered from the whole of the respiratory tract, but not from the organs. A guinea-pig inoculated with a culture from the trachea died in three days; the subcutaneous oedematous fluid showed the same characteristic bacillus, and cultivations were obtained from this and also from the blood. Guinea-pigs were also inoculated with cultures from the nasal mucous membrane, and from the conjunctivae with similar results. Other guinea-pigs were inoculated with the subcutaneous oedematous fluid and these died under similar conditions. Three guinea-pigs which had four days previously received an inoculation of 5 c.c. of blood serum from horse 1 were inoculated with a suspension of an agar plate culture of the nasal mucous membrane; three fresh guinea-pigs were also inoculated with a similar amount as controls. One of the controls died in two days under similar conditions as the above.

Horse 7. Suffering badly, cultures were made from the conjunctival discharge, and showed the characteristic bacillus. Afterwards, when the horse died, cultures were made from the nasal mucous membrane, bronchus of healthy lung, bronchus of pneumonic lung, and from the pneumonic lung substance. The same bacillus

was obtained from each specimen. The solid lung and its bronchus gave also a number of small colonies which proved to be a streptococcus, but it was without effect when inoculated into a guinea-pig.

Summary. In the above observations we found a small bacillus usually growing in pairs on artificial media, which from its reactions and cultures would appear to belong to the coli group. The whole respiratory tract of the affected animals seem to be infected, but from the fact that animals may die of the disease without showing 'pink-eye,' it is probable that the infection starts in the respiratory tract, and invades the conjunctiva through the nasal duct.

This bacillus is usually virulent to guinea-pigs, causing death in two to three days. From the fact that it was recovered constantly from the respiratory tract and conjunctiva of dead or diseased animals, there is strong presumptive evidence that it was the cause of the disease, but we have no experimental proof that it caused the typical disease under artificial inoculation.

The case would appear to be similar to those pathological conditions where the *B. coli* is found to have taken on an increased growth and virulence, as in some forms of enteritis.

REPORT OF THE LIBRARIAN

REPORT OF THE LIBRARIAN

The past year has been one of remarkable development for the Library. By the generosity of the Rev. S. A. THOMPSON YATES we have been able to acquire, from their commencement, the following valuable periodicals :—

Archiv f. Pathologische Anatomie (Virchow's Archiv)
Archiv f. Hygiene
Archiv f. d. gesammte Physiologie (Pflüger's Archiv)
Archives de Pathologie et Physiologie (Brown- Séquard's Archives)
Archiv f. exper. Pathologie, etc. (Schmiedeberg's Archiv)
Beiträge z. Patholog. Anat. (Ziegler's Beiträge)
Centralblatt f. Bakteriologie. Pts. I and II
Centralblatt f. allg. Pathologie
Centralblatt f. Physiologie
Zeitschrift f. Hygiene, etc.
Zeitschrift f. Physiologische Chemie (Hoppe-Seyler)

together with several others noted below.

Moreover, by an arrangement with the Library Committee it was agreed to transfer certain periodicals from the Tate Library to the Departmental Library, on the condition that the previous numbers of those which were not complete should be purchased by the Department. In this way the Departmental Library has become fairly comprehensive in the literature of Bacteriology, Pathology, Physiology, Hygiene, and Neurology. There are still some lamentable gaps, but at present there are no funds by which they may be filled.

The number of exchanges has increased ; to several editors of old standing periodicals we owe thanks for their generous readiness to exchange.

ALBERT S. GRÜNBAUM

HON. LIBRARIAN

LIST OF PERIODICALS TAKEN BY DEPARTMENTAL LIBRARY

- (C) Annales de l'Institut Pasteur
- (C) Arbeiten a.d. Kaiserl. Gesundheitsamte
- (C) Archiv f. Pathologische Anatomie u. Physiologie
Archives italiennes de biologie
- (E) (C) Archiv f. Hygiene
Archives orientales de médecine et de chirurgie
Archiv f. experimentelle Pathologie u. Pharmakologie
- (C) Archives de Physiologie et Pathologie générale
- (C) Archiv f.d. gesammte Physiologie
- (C) Archiv f. Psychiatrie u. Nervenkrankheiten
- (C) Archiv f. wissenschaftliche u. praktische Thierheilkunde
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- (C) Beiträge z. pathologischen Anatomie u.z. allgemeinen Pathologie
- (P) Brain
- (P) British Medical Journal
- (E) Bulletin of the Johns Hopkins Hospital
- (C) Centralblatt f. Bakteriologie : I Abtheilung
- (C) Centralblatt f. Bakteriologie : II Abtheilung
- (C) Centralblatt f. allgemeine Pathologie u. pathologische Anatomie
- (C) Centralblatt f. Physiologie
- (E) (C) Deutsche Vierteljahrsschrift f. öffentliche Gesundheitspflege
Deutsche Medicinische Wochenschrift
- (C) Deutsche Zeitschrift f. Nervenheilkunde
- (E) Gaceta Médica Catalana
- (E) Guy's Hospital Report
- (C) Hygienische Rundschau
- (E) Indian Medical Gazette
- (E) Johns Hopkins Hospital Report
- (E) Journal of Experimental Medicine
- (C) Journal of Hygiene
- (C) Journal of Pathology
- (P) Journal of Physiology
- (E) Journal of State Medicine
- (E) Journal of Tropical Medicine
- (E) Journal of the Boston Society of Medical Sciences
- (E) Journal of Balneology and Climatology
- (C) Journal de Physiologie et de Pathologie générale
Lancet

- Neurologisches Centralblatt
(E) Il Poloclinico
(P) La Presse Médicale
(E) Public Health
(E) Revista de Medicina Tropical
(E) Revista medica de S. Paulo
(P) Sanitary Inspector
(E) St. Thomas's Hospital Report
(E) University of Pennsylvania Medical Bulletin
(E) Upsala Läkareförenings Förhandlingar
(C) Veröffentlichungen d. Kaiserl. Gesundheitsamte
(E) Vierteljahrschrift f. gerichtliche Medicin u. öffentliches Sanitätswesen
(C) Zeitschrift f. Fleisch-u.Milchhygiene
(C) Zeitschrift f. Hygiene u. Infektionskrankheiten
(C) Zeitschrift f. Physiologische Chemie
(C) Zeitschrift f. Biologie

C = from the commencement

E = exchanged

P = presented

THOMPSON YATES LABORATORIES
REPORT

THE THOMPSON YATES LABORATORIES REPORT

EDITED BY
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WITH ILLUSTRATIONS AND PLATES

VOL. IV. PART II

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CONTENTS

	PAGE
The Injury Current of Nerve : The Key to its Physical Structure	<i>J. S. Macdonald</i> 213
Observations on the Physiology of the Cerebral Cortex of some of the Higher Apes	<i>A. S. F. Grünbaum</i> 351 <i>C. S. Sherrington</i>
Tubercular Expectoration in Public Thoroughfares : An Experimental Enquiry. First Communication	<i>H. E. Annett</i> 359
Pseudo Actinomyces of the Udder of the Cow	<i>Rubert Boyce</i> 371
An Isolated Case of Plague	<i>A. Stanley Griffith</i> 379
A New Pathogenic Bacillus Isolated from a Case Diagnosed as Typhoid Fever, with a Summary of Fourteen Similar Cases Hitherto Reported	<i>Edward H. Hume</i> 385
Note upon Fungus Deposits in Unfiltered Water Mains	<i>Rubert Boyce</i> 409
Sulphide Producing Organisms	<i>E. N. Coutts</i> 417
A New Nitrometer for the Clinical Estimation of Urea by the Hypobromite Process	<i>W. Gordon Little</i> 433
Extensive Focal Necrosis of the Liver in Typhoid	<i>E. E. Glynn</i> 441
Multiple Aneurisms of the Aorta	<i>John Hill Abram</i> 449 <i>Lyn Dimond</i>
Preliminary Note upon a Trypanosome Occurring in the Blood of Man.	<i>J. Everett Dutton</i> 455
Quelques Notes sur les Embryons de Strongyloides Intestinalis et leur Pénétration par la Peau	<i>P. Van Durme</i> 471
The Report of the Yellow Fever Expedition	<i>H. E. Durham</i> 485

THE INJURY CURRENT OF NERVE



CONTENTS

	PAGE
Preface	213
Historical Section	215
Note on the Physical Theory of Nerve Function	225
The Detailed Examination of the Phenomenon	229
Changes in the Phenomenon Produced by the Conditions of an Experiment	246
Measurements of the Electrical Conductivity of Nerve	258
The Physical Structure of the Nerve	266
Effect on the Phenomenon Produced by an Immersion of the Nerve in Water	273
Also upon the Phenomenon as Found in Degenerated Nerve	282
The Effect Produced by Immersion in Solutions of Electrolytes	288
Also upon Nerve which has been Removed from an Animal sometime after Death	300
The Action of Solutions of Electrolytes Studied Quantitatively	305
The 'Concentration Law'	310
The 'Concentration Law' in the Special Case of Solutions of Potassium Chloride	326
The Conductivity of the Internal Solution	340
General Conclusions	344

ERRATA

- P. 264, line 34. For 'conductor' read 'conduction.'
- P. 309, line 10. For 'simply' read 'simple.'
- P. 346, line 14. For 'organic' read 'inorganic.'
- P. 359, in reference (2). For '*Theirkeilkunde*' read '*Thierheilkunde*.'
- P. 360, in reference (1). Read *Zeitsch. f. Hygiene*.
- P. 361. For 'NIELSEN's' read 'NEELSON's.'
- P. 371, footnote. For '*Klinischer*' read '*Klinische*.'
- P. 409. For '*Sphaerolitus*' read '*Sphaerotilus*.'
- P. 414, line 20. For 'pressure' read 'presence.'
- P. 444, last line. For 'WELSH' read 'WELCH.'
- P. 446, last line. For 'WELSCH' read 'WELCH.'
- P. 461, line 13. For '*Hoemoglobin*' read '*Haemoglobin*.'
- P. 461, line 13. For 'Gower's' read 'Gowers'.'
- P. 461, line 6 from bottom. For 'LABBE' read 'LABBÉ.'
- P. 465, line 7 from bottom. For '*T. Brucei*' read '*T. brucei*.'
- P. 499, in footnote. For 'SCHWALBS' read 'SCHWALBE.'
- P. 503, in footnote. For '*Aufklärung*' read '*Aufklärung*.'
- P. 528, chart opposite, in Case 1. For 'Nov.' read 'SEP.'



THE INJURY CURRENT OF NERVE

BY J. S. MACDONALD

PREFACE

IN justification of this attempt to secure a hearing for an already elaborately-handled theme, it may be pardonable to emphasize the importance of a very obvious fact, that the joint work of chemists and physicists has made the present time extremely opportune for the conduction and consideration of such research.

It may be said that this phenomenon of the injury current was most exhaustively examined at a time when physical science was not ripe to deal with such possibilities, and that the physiologist was obliged at great inconvenience to grope for correlated physical data; whereas, at the present time, such a mass of the required information has been collected, codified, and arranged according to simple and sufficing explanatory hypotheses by the physicist, that physiological research of this kind can with great advantage be carried out upon easily anticipated lines.

It may, on the other hand, be said that at the present date it is much more necessary to apologize for the carrying out of any study of electrical phenomena occurring in animal tissues, and especially such as are consequent upon injury, than to be imbued with a sense of their importance; for it is now realized that every process of diffusion occurring between solutions of electrolytes, such as solutions of inorganic salts, is a probable source of electrical phenomena, and also that every injury is necessarily succeeded by processes of diffusion following the destruction of pre-existing barriers. It is, for example, an absolutely certain prediction that differences of potential must be found between electrodes placed upon the external and internal surfaces of glandular organs, upon which it is an otherwise amply determined fact that solutions of inorganic salts are present differing in concentration or in nature in these two positions. And again, when it is otherwise known that stimulation of nerves leading to these structures causes a further difference to arise between the two solutions, it is certain that new differences of potential will arise as the result of such stimulation. In such cases it may seem doubtful whether the information gained from an examination of differences of potential can be of interest in comparison with the more direct information attainable by other means.

In the case of nerve no such apology is needed, for no other means have yet been found for detecting and measuring its intrinsic changes, even if these can finally be shewn to be of chemical rank ; nor can the study of the injury current be exempted from this claim.

There still remains the great probability that a nervous impulse may be a change propagated by electrical agency, and even in its essential nature an electrical phenomenon ; a travelling and temporary dislocation of pre-existing discrete particles, and not a travelling process producing new and differently gifted particles from the old.

If so, it is as solutions of electrolytes confined to minute cylinders that nerve fibres have a most important interest ; and yet the characteristics of these solutions are beyond the reach of methods of ordinary chemical investigation.

Therefore, any method which promises to reveal, even in an indirect way, the nature and concentration of these solutions, should be considered deserving of immediate and zealous application.

The investigation of the injury current stands alone in offering such possibilities ; and its diligent prosecution is not a matter for apology, nor even of secondary though legitimate importance, but is the only means by which a knowledge of the fundamental structure of the nerve can be obtained.

HISTORICAL SECTION

The occurrence and distribution of differences of potential between points upon the surface of excised nerve, was discovered in 1842 by DU BOIS REYMOND, published in 1843,¹ and described in an exhaustive manner in his first collection of researches² in 1849. The general account given by him of this phenomenon provides one with an example of the possible truth and completeness of experimental observation. Rapidly as the research must have been carried through, for it forms only an incident in a larger theme, the statement is a perfect one, and an intimate acquaintance with the phenomenon reveals the amount of observation concealed behind each one of its carefully considered sentences. The salient points were gathered into three generalizations, the well-known *laws of the nerve current*. They define the spatial distribution of points upon the surface of the nerve, between which the differences of potential were (1) large, (2) small, (3) non-existent.

These laws describe the ideal case, which it is carefully noted was rarely, if ever, obtained, and in their application to the facts of any single experiment are stated generally to require modification. The most important modifications being due to the occurrence of a potential difference between the two cross sections and to the dislocation of the equatorial point from the mid point of the nerve.

MORGAN, in 1863,³ pursued the investigation one step further, and demonstrated the existence of a nerve current in minutely thin longitudinal fragments of nerve trunk, obtained for the experiment in a manner described by HARLESS⁴. He also noted the presence of 'weak longitudinal currents' in these fragments, and ascertained the symmetrical arrangement of potential distribution round an equatorial point.

The remaining work which has been undertaken in this subject has, to a large extent, only been concerned with the comparison of results obtained from various nervous tissues, and with modifications devised to test theories of its mode of origin, or with results obtained in abnormal or pathological conditions of the nerve.

The relation between the conditions thus found upon the surface of the nerve trunk, and its microscopical fragments, and the conditions of greater interest and importance legitimately inferred from a consideration of these to exist in the more

1. Du Bois Reymond, *Poggendorf Annal.* Bd. LXVIII. January, 1843. 2. *Untersuchungen.* II (1), p. 262.
3. Charles E. Morgan, *Reicherts Archiv.*, 1863, p. 340; also *Electrophysiology and Therapeutics.* New York, 1868, p. 465.
4. E. Harless, *Abhandl. Konigl. Bayerisch. Akad. d. Wiss.* 2nd Class, Bd. VIII. Abth. II, p. 539.

deeply-situated nerve fibres, was elaborately investigated by DU BOIS REYMOND.¹ His inferences strengthened by the mathematical work and physical experiments of HELMHOLTZ² explained the complex distribution of potential differences upon the nerve trunk surface, as due to a simple distribution of oppositely electrically charged surfaces upon the individual nerve fibres. The zinc-copper model exemplifies these inferences, and its composition is a standing witness to the fact that the complex superficial conditions are, to a great extent, the outcome of an examination of the nerve trunk when longitudinally isolated from the surrounding structures, and examined in air. The complex condition then found upon the surface of the nerve trunk is new, is not the same as the conditions existent upon its surface when clothed in the neighbouring tissues, nor is it the same as the conditions existent upon the important elements of its structure.

In concentrating attention upon the individual nerve fibre and its physical structure, and after a laborious investigation of surface distribution, legitimately delving from this the essential nature of the problem as it affected the individual nerve fibre, DU BOIS REYMOND may be said to have not only discovered the 'apparent' but also the 'real' phenomenon. The whole longitudinal surface of the individual nerve fibre is probably equally positive, the whole transverse surface uniformly negative. The gradual transition, which apparently takes place in the surface conditions as we pass from the cross section in towards the unbroken nerve fibre, has probably no parallel in a varying state of the nerve fibre. The nerve fibre at its cut end is negative. The longitudinal surface in the neighbourhood of this cut end appears less positive than the remainder of the longitudinal surface, simply because of its physical juxtaposition to this negative surface.

SECTION I

EXPLANATORY THEORIES. DU BOIS REYMOND

Of these facts and legitimate inferences, various explanations have been offered. DU BOIS REYMOND based his explanation entirely upon the constitution of one primarily important structural element of the nerve fibre. He used, in fact, the data obtained from observations upon the manner of origination and conduction of electrical currents by the nerve as a base from which to obtain knowledge of the main physical characteristics of the structure of the nerve fibre. It is a matter for regret that he did not sufficiently recognize in the nerve fibre the existence of not one but several structures of possible importance. It is true that before committing himself finally to such a position he was careful to examine the other two lines upon

1. Du Bois Reymond, *Untersuchungen*. Bd. I, p. 672. 1849. The original work was done upon muscle but confirmed for nerve.

2. Helmholtz, *Pogg. Ann*, Bd. LXXXIX, p. 212. 1853. For a full account see also A. Fick, *Die Medizinische Physik*, Braunschweig, 1858; or C. Morgan, *Electro-physiology*, New York, 1867.

which an explanation might be sought, namely (1) a difference between the chemical constitution and physical characteristics of the molecules forming the longitudinal surface and transverse section respectively, due to secondary changes (acidity, etc.) at the transverse section;¹ and (2) a pre-existing difference between the nerve substance proper and its sheath.²

The first of these two rejected alternatives is closely akin to HERMANN'S 'alteration' theory, and was dismissed because of the apparent disproportion in extent between the phenomenon to be explained and the chemical change which could otherwise be demonstrated. The second alternative is as closely akin to GRÜNHAGEN'S theory, and was dismissed because of an apparently decisive contradictory experiment (see later).

Having once selected a line of explanation, his further developments of it were foreordained by the scientific attitude of the time, which translated wholesale the attributes discoverable within and at the surface of a mass of homogeneous material to each of the individual molecules of which it was formed. The peripolar³ molecule was a minute zinc-copper model unit, and its conception was then the necessary outcome of the legitimately formed belief that the nerve or muscle fibre was alone the physical structure upon which the phenomenon depended, and that this could practically be treated as a homogeneous structure.

Similarly when DU BOIS REYMOND discovered that under certain circumstances the muscle fibre could not be treated as homogeneous, and that the condition then present in localized portions of the fibre prevented the demonstration of the phenomenon, it was only possible to consider that such localized portions (parelectronic layer)⁴ offered an opposing electromotive force: it not being known that neutral 'membranes' might eliminate the display of electrical differences by offering an impermeable obstacle to the movement of diffusing particles, and that there was, therefore, no necessity to credit such obstacles as were discovered with what one might call 'electromotive functions.'

NOTE ON THE PARELECTRONIC LAYER

Du Bois REYMOND'S conception of the parelectronic layer has been misunderstood, and even ridiculed, as if invented when the trend of a controversy compelled him to meet new evidence and satisfy impossible claims upon his original 'peripolar molecule' conception. It may therefore be of some advantage to consider the following quotation taken from a book published in 1852, and, therefore, fifteen years before the appearance of the 'alteration theory':—

H. BENCE JONES, *Animal Electricity* (being an abstract of the discoveries of EMIL DU BOIS REYMOND), page 116; published by Churchill, London, 1852—'The current obtained from the longitudinal section and the natural transverse section is seldom, if ever, so strong as the current obtained from the longitudinal

1. *Monatsberichte d. Königl. Akad.*, 2 Berlin, 1859, p. 288. Republished in Du Bois Reymond's *Gesammte Abhandl.*, II, I, 5.

'After my discovery in 1842 of the muscle current, naturally one of my first experiments was undertaken to discover whether the longitudinal surface and the artificial cross section of muscle possessed different reactions.'

2. *Untersuchungen*, I, 558. 1848.

3. Du Bois Reymond, *Untersuchungen*, I, p. 561, 1848; see also C. Morgan, *Electrophysiology*, etc., p. 279, 1867.

4. Du Bois Reymond, *Untersuchungen*, II, p. 39; or C. Morgan, *loc. cit.*, pp. 294-309.

section and the artificial transverse section. Very often, indeed, the former appears incomparably weaker; and by keeping the frogs for twenty-four hours at least at a temperature of 32° F. (0° C.) it is possible wholly to deprive the natural transverse section of its negative power when it is included in a circuit with the longitudinal section. But even the direction of the current can be reversed by means of cold. Whichever of these various modifications of the electric power of the natural transverse section may prevail, the usual current immediately appears when this section is injured in any way, so as to deprive an extremely thin layer of its vital properties, and thereby of its electromotive action. . . . Since the layer of muscular substance on the natural transverse section tends to reverse the laws of the muscular current, Du Bois REYMOND proposes to call it the *parelectronomic* layer (from *παράνομος* contrary to the law), and he likewise calls that the *parelectronomic* state of the muscle, in which the muscle, in consequence of the *parelectronomic* layer having the intensity of its action increased, either appears inactive, or even becomes inactive in the negative direction,' etc.

SECTION II. HERMANN

Beginning in 1867, HERMANN¹ produced a series of papers which proved that the phenomenon of the current of rest could not be demonstrated in uninjured tissue, and that injury, sometimes of an unsuspected kind, was an essential factor in its production. This proof he accompanied by an emphatic and controversial insistence upon the importance of the secondary results of injury, and in deference to his views the phenomenon has changed name, becoming the current of injury and subsequently the demarcation current. The extreme emphasis laid by him upon the secondary results of injury has since been abandoned,* and in 1898 he is found deliberating between his own 'alteration theory,' which embodies these views, and a 'pre-existence' theory belonging to that general class of theories against which he so strenuously contended.

It has been said that Du Bois REYMOND's 'peripolar molecule' was formulated upon a basis of assumption, that the matter giving rise to the phenomenon was pre-existent and homogeneous. It must also be said that HERMANN's 'alteration theory' is confessedly based upon an assumption,² which must be considered, too, as possibly obnoxious; for it is an assumption of knowledge which is, in this case, unfortunately not otherwise attainable.

'Let us suppose that the dying substance is negative to the living, then all these phenomena are explained.'³

The acceptance, even temporarily, of such a postulate as this is equivalent to the surrender of a legitimate spirit of enquiry, and is impossible to a mind seeking to unravel the intricacies of 'vital' phenomena by gratefully received details of chemical

1. Hermann, *Untersuchungen zur Physiologie der Muskeln und Nerven*. Berlin, 1867-1868.

* See later, p. 223.

2. Hermann, *Pflügers Archiv*, 1898. LXXI, p. 299.

3. Hermann, *Handbuch*. Th. I, p. 235.

NOTE.—Hermann's alteration theory is expressed in terms of vital states. For a reduction of these to the terms of chemical and electrochemical nomenclature—see (1) Bernstein, *Pflügers Archiv*, 1897, who evolves a theory which, superficially similar to Du Bois Reymond's in that it is a molecular one, nevertheless involves a chemical difference at the seat of injury and is therefore similar to Hermann's; (2) Tschagowetz, 1897, *Ztsch d. russ. Gesellsch f. phys. Chem.*, XXVII, 5, p. 430; (3) Max Oker Blom, 1901, *Pflügers Archiv*, LXXXIV, p. 191.

composition and physical conditions. 'Vital' phenomena, not otherwise known to exist, are practically created to explain an interesting physical phenomenon; and are suitably arranged in the course of the nerve fibre in a manner which is not open to corroboration.

TO DU BOIS REYMOND such an acceptance would have involved not only the abandonment of a particular theory, but also the relegation of his whole subject to the regions of empirical symbolism. It is not surprising, therefore, that immediately the theory was promulgated he entered the field against it.¹

The extent of the controversy is well known. The experimental data collected during its prosecution will, doubtless, be remembered, when the words which hurled them into the possession of contemporary science have long been forgotten; but DU BOIS REYMOND will then have been reinstated as the founder of a scientific method of physiological research, and will thus have a more honoured remembrance than as the discoverer of a fact or a series of facts, or as the defender of a perhaps too confidently-held position. In the meantime the indirect outcome of the controversy and of its lessons has been a philosophy, which sees in comparative 'negativity' a long sought-for rule, by which to measure accurately the relative intensity of life in two contrasted situations.² In repose positive, in activity negative, only given a standard of candle power the value of any vital spark could immediately be assessed by the galvanometer.

In 1877, HERMANN³ published experimental data, which very largely moulded the general opinion as expressed in contemporary and in subsequent text-books. These data, obtained by the use of his 'fall rheotome,' proved that the current of injury did not traverse the galvanometer circuit in fully developed strength for a short interval ($\cdot 0025''$) after the occurrence of the injury.

The fact was advanced as conclusive evidence against all 'pre-existence' theories; so that DU BOIS REYMOND's and GRÜNHAGEN's and every similar point of view must be abandoned. The conclusion was practically accepted, and has been followed by consequences of importance.

To regard the injury current as possibly the outcome of pre-existing conditions is to regard it as a phenomenon of primary interest, as, in fact, a possible key to the desired knowledge of the physical structure of the nerve, and, therefore, to the knowledge of the possibilities of the meaning of its physical change during function.

The fall rheotome experiments temporarily removed any general confidence which had been maintained in such a possibility, and, as a consequence, deposed the phenomenon to a rank of secondary importance.

The delay measured by means of the fall rheotome is, however, by no means conclusive evidence of a time spent in the initiation of chemical or of vital change.

1. Du Bois Réymond, *Monatsberichte d. Königl. Akad. d. Wiss.*, Berlin, 1867, p. 597; reprinted in Du Bois Reymond's *Gesammte Abhandl.*, II, p. 319.

2. E. Hering, *Lotos*, Prague, 1888; translated 'Brain, 1897, p. 232.'

3. Hermann. *Pflüger's Archiv*, XV., p. 191. 1877. Experiments upon muscle.

There have first to be considered such factors as polarization, self-induction, and, not least, the fact that chemical substances in solution only give rise secondarily, by processes of diffusion, to electrical phenomena. It is not, therefore, surprising that HERMANN, in 1898, is somewhat less confident of the truth of the alteration theory than in 1867. Its establishment as the prevalent hypothesis has rested not so much upon evidence adduced directly to support it as upon evidence which temporarily cut the ground from beneath the feet of its opponents.

It is necessary to realize this fact, and to remember the powers of adverse criticism which indefatigable research and a skilful handling of physical and mathematical detail, and the position of authority which the victory in this controversy and the esteem in which his other contributions to physiological literature are held, have conferred upon HERMANN.

SECTION III. GRÜNHAGEN

GRÜNHAGEN¹ in 1866, a year before HERMANN's promulgation of the alteration theory, advanced a theory, like DU BOIS REYMOND's in so far that the conditions of importance were assumed to be 'pre-existent' in the nerve before the injury, but unlike it in that it involved, as prime factors, conditions existent in the ensheathing tissues of the nerve trunk, as well as in the physiologically important elements of structure. GRÜNHAGEN's advance to this position was due to a consideration of the histological structure of the nerve, and to experiments undertaken by him in which he brought to notice circumstances of importance hitherto unconsidered in this problem: for he discovered in combinations of various solutions and 'membranes' a possible source of definitely-directed electrical currents.

The first 'membranes' used by him were porous clay pots, and his explanation of the value of the whole combination was given in terms of the capillary pores of these structures, and of the QUINCKE² 'diaphragm currents' arising from the passage of water through them. Further examination revealed other possible explanations, and in 1874³ one finds him as the discoverer of a 'new kind of electrical current' as distinguished from currents thus originated by the passage of water. This new source of electromotive force was, undoubtedly, what would now be described as a partially permeable 'membrane' separating solutions of electrolytes. Of the efficacy of such combinations there now seems little room for doubt, as also of the similar ones examined previously by BUFF in 1854, and ridiculed by HERMANN in 1871 (see later). There can also be little doubt but that GRÜNHAGEN insisted upon the importance of the membrane in the combinations described by him, and this point must be remembered in considering HERMANN's criticism. HERMANN, one must

1. Grünhagen, *Königsberger Med. Jahrb.* IV, p. 199. 1886.

2. Quincke, *Pogg. Ann.* CVII, p. 37, CX, p. 56; also paper by Kunkel, *Pflüger's Archiv.*, XXV, p. 342. 1883.

3. Grünhagen, *Pflüger's Archiv.*, VIII, p. 573. 1874.

also remember, at first regarded the axis cylinder as an artefact,¹ and has, until quite recently, stoutly resisted the attempt to consider the axis cylinder and myelin sheath as necessarily the possessors of different physical characteristics. HERMANN has wrongly² depreciated³ the importance of a dividing membrane in deciding the occurrence of polarization phenomena, and in his polarization experiments the presence of such membranes was rigidly excluded.⁴

There are many grounds, therefore, to justify one in considering that HERMANN did not appreciate the important part played in GRÜNHAGEN's models by the dividing membrane, nor the extreme probability that analogous parts were really to be found in the nerve.

In HERMANN's Handbook, 1879 (Bd. I, p. 234), we find the arguments which the propounder of the 'alteration theory' uses to dismiss GRÜNHAGEN's theory based upon the assumption of pre-existing heterogeneous structures in the nerve. It is taken that its most decisive refutation is to be found in the manner in which it leaves unexplained, or badly explained, the correlated phenomenon of the action current. It may, indeed, be true that the particular explanation of the action current which GRÜNHAGEN advocated may be insufficient or even of little interest. It may be true that the alterations of resistance which he invoked may not occur, nor even if occurring may fail to provide a direct explanation of this other phenomenon. But it is equally true that HERMANN's own explanation of the action current almost necessarily entails the occurrence of alterations of resistance, and that the failure to discover such alterations would also greatly militate against this.⁵

But, disregarding the particular explanation of the action current offered by GRÜNHAGEN, and also the possible non-coincidence of the two phenomena of action and injury current, it can hardly be said that the physical structures invoked by GRÜNHAGEN are such as would by their arrangement prevent the development of the current of action; for these are just the structures upon which now, and with a great shew of reason, the attempt is being made to explain this phenomenon (BORUTTAU, STRONG, etc.).⁶

In the second place, HERMANN uses DU BOIS REYMOND's⁷ contra indicating experiment, which was undertaken by DU BOIS REYMOND as a crucial test of a tentative hypothesis he temporarily advanced, and has been accorded a 'classical position' by HERMANN until recently. It is apparent upon consideration that this test, whereas

1. Hermann, *Pflügers Archiv.*, LXXI, p. 283. 1898.

2. Nernst quoted by Boruttau, *Pflügers Archiv.*, LXXVI, p. 626.

3. Hermann, *Pflügers Archiv.*, VI, p. 342.

4. Hermann, *Nachrichten v. d. Göttinger Gesell. d. Wiss.*, pp. 326-347. 1887.

5. The alteration theory sees in both dying and active tissue the presence of a similar state of activity accompanied by a similar chemical change. If, as is most usually supposed, this chemical change involves the breaking down of complex organic compounds, and the separation from them of simple dissociation products, it almost certainly follows that non-electrolytes are broken down into electrolytes, and so cause alteration in resistance. In fact, upon this assumption a simple explanation of both action current and injury current might readily be produced, the membrane by its selective influence upon the velocity of positive and negative ions might lead in the resulting diffusion processes to electrical phenomena.

6. Boruttau, Strong, *loc. cit.* Cremer, *Ztschrift für Biologie*, pp. 37, 550.

7. Du Bois Reymond, 1848, *Untersuchungen*, I, p. 558; also a repetition of the same experiment for nerve, C. Morgan, *Electrophysiology*, p. 465.

valid and sound as applied to DU BOIS REYMOND's idea, loses all interest when applied to GRÜNHAGEN's.

DU BOIS REYMOND's experiment was as follows :—The muscle was torn into minute strips so as to obtain longitudinal fragments containing only a few muscle fibres. One of these longitudinal strips was laid upon one electrode; another was placed so as with its transverse section to touch the longitudinal surface of the first, and with its own longitudinal surface upon another electrode. *The normal 'current of rest' of the second strip was observed to be present.*

The idea which DU BOIS REYMOND tested by this experiment was the possible presence of two heterogeneous structures in the muscle fibre, two physical structures capable of causing an electrical current, when in contact, as, for instance, do copper and zinc. Such a probability was forced upon him by the histological distinction between the sarcolemma and the contents of the muscle fibre. The results of the experiment given above were taken, and, it is obvious, legitimately so, as conclusive evidence against the possibility; for, as DU BOIS REYMOND said then, the structures were arranged as follows :—

FIRST FIBRE
┌───────────┐
Sarcolemma. Contents. Sarcolemma.

SECOND FIBRE
┌───────────┐
Contents. Sarcolemma.

The arrangement is thus symmetrical, and is incapable of giving rise to an electrical current if the assumption tested were true, as, for instance, would also be the case with the similar arrangement—

Copper. Zinc. Copper. Zinc. Copper.

Since the experiment did, however, result in the exhibition of the usual current, the assumption and this method of regarding the arrangement of structures in the experiment were necessarily excluded.

But this test is meaningless when applied to GRÜNHAGEN's theory, as was done by HERMANN, for the structures as seen in its light are as follows :—

FIRST FIBRE
┌───────────┐
Nutritive fluid. Membrane. Contents.

SECOND FIBRE
┌──────────────────────────┐
Nutritive fluid. Membrane. Contents. Membrane. Nutritive fluid.

The arrangement is here seen in its true light as an asymmetrical one, *and as necessarily productive of an electrical current*, and the discovery of such a current in the experiment is, therefore, a circumstance capable of anticipation. The application of such a test to this idea is, therefore, without point, and in its result a confirmation and not a contradiction of the hypothesis.

After reviewing the evidence which influenced HERMANN in rejecting GRÜNHAGEN'S theory, including the two points just dealt with, and also the results of the fall rheotome experiments previously considered, we may pass to consider HERMANN'S position in 1898.¹

We find the sufficiency of the 'alteration' theory unabandoned.² This is but natural. But we find HERMANN very seriously considering the claims of a pre-existence theory based upon the assumption of two heterogeneous physical structures in the nerve fibre.

We find, in fact, HERMANN coming forward with proof of the existence of such a difference between the sheath and contents of the nerve fibre, as would, if conclusive, fully justify an *à priori* plea for the formation of such a theory. And the theory which HERMANN thus revives is not GRÜNHAGEN'S, but is DU BOIS REYMOND'S tentative hypothesis.

It is necessary, therefore, to turn again to DU BOIS REYMOND'S crucial test as criticized both in 1879 and in 1898 by HERMANN; in fact, as necessarily unfavourably criticized by him 1867, for the complete refutation of this supposition could alone have prepared the way for the promulgation of the alteration theory.

Du Bois REYMOND thought that he was arranging symmetrically the muscle fibre contents and sheath, and paid no attention to the 'nutritive solution' bathing these structures, for he regarded it merely as a neutral conducting medium, and did not know that it might play an active part in the production of the phenomenon.

HERMANN saw³ and sees⁴ that the arrangement is asymmetrical, and that DU BOIS REYMOND'S test is unconvincing, but he does not see the asymmetry as given above, but in the light of the vital states postulated by the 'alteration theory'

FIRST FIBRE

Sarcolemma. Normal contents. Sarcolemma.

SECOND FIBRE

Dying contents. Normal contents. Sarcolemma.

1. Hermann, *Pflüger's Archiv.*, LXX, p. 523, 1898. The experiments related by Hermann in this paper and in another (*Pflüger*, LXVII, p. 240) are of a somewhat remarkable kind. The passage of the strong currents through microscopical fragments of nerve are observed to produce protrusions of the myelin from the nerve fibre. Upon this bodily movement of tube contents upon the tube, arguments are based which lead Hermann to consider the essential physical difference between these structures.

2. Hermann, *loc. cit.*; also *Pflüger's Archiv.*, LXXI, p. 296 *et seq.*, 1898.

3. Hermann, *Handbuch*, *loc. cit.* 4. Hermann, *Pflüger's Archiv.*, LXX, *loc. cit.*

That is to say, that in attempting to decide between the two theories, the alteration theory and this pre-existence theory, he does not place them in clearly alternative positions, but considers the second as a corollary to the first. No proofs have ever been produced of the existence of a dying condition at the cut end of a nerve or even of a localised chemical change. Both may, indeed, exist, but the primary reason for considering the possibility of such a state is that HERMANN propounded the alteration theory. *Hermann is, therefore, considering the pre-existence theory from the point of view of the teachings of the alteration theory, and not as a quite separate and distinct possibility, and must have done so from the first.*

The general impression left upon one's mind is that GRÜNHAGEN's theory has never met with the consideration nor with the acute criticism which it deserved as a logical deduction from a valuable appreciation of the relative values of the structures of the nerves.

NOTE

GRÜNHAGEN's theory in its most acceptable form was completely anticipated by BUFF in 1854, although applied to electrical currents obtainable from plant tissues, and not from muscle or nerve. (*Ann. d. Chem. u. Pharm.*, Bd. 89, 76. 1854.) The currents obtained were explained as the result of—

- (1) A negativity of the sap ;
- (2) A positivity of the surface water ;
- (3) A sharp delimitation of the two solutions by the epidermis.

BUFF also supported his conclusions by physical experiments of interest.

JURGENSEN, in 1861, also working with plants, came to very similar conclusions supported by similar experiments. (*Studien. d. Physiol. Institut. zu Breslau*, I, 87-109. Leipzig, 1861.

J. REINKE, in 1882, also discussing plant currents, refused to admit an explanation couched in terms of vital states, and anticipates a possible explanation in the complex arrangement of moist conductors contained in plants. (*Pflüger's Archiv.*, XXVIII, 143, 1882.)

J. S. MACDONALD, in 1900, produced certain evidence obtained from mammalian nerve, and considered this to be in support of a similar theory.

BUFF's and JURGENSEN's conclusions were adversely criticized by HERMANN in 1871. He also criticized the value of their physical experiments, performing similar experiments with uncertain results. The value of BUFF's, JURGENSEN's, and GRÜNHAGEN's physical experiments are, however, now easily assessed by an appeal to the new subject of electrochemistry. (HERMANN, *Pflüger's Archiv.*, IV, p. 148, 1871.

SECTION IV. BORUTTAU

DU BOIS REYMOND used the current of rest as a phenomenon from which to extract a practical conception of the physical structures of the nerve, and proceeded with this structure in his mind to explain the other electrical phenomena which could be demonstrated in it. BORUTTAU has used the polarization phenomena in a similar manner. The polarization phenomena entail a 'core model' structure for the nerve, and have been held to do so by several investigators. Granted the 'core model' structure of nerve, all the other electrical phenomena of nerve are stated as explained by it.

It has been stated previously (see note, p. 221) that a 'core model' structure (such as was assumed by GRÜNHAGEN) might be used to explain the electrical phenomena of the 'action current,' even if they were the secondary consequences of chemical change taking place in the axis cylinder. This possibility has been considered by BORUTTAU, forming, as it does, one of the two only probable lines upon which this phenomenon can be investigated and perhaps explained. But he has taken a greater interest in the other possible explanation, and has sought confirmative suggestions from experiments upon 'core models,' and has also sought confirmatory facts from experiments upon nerve.

According to this second explanation, the nerve has not only a core model structure, but also that structure is just of the kind required to act as the purely physical conductor of electrical change, and to conveniently transmit energy from point to point without involving the development of new sources of energy (chemical changes) in its line of progress. Such a conception of the arrangement of structures in the nerve has also been frequently debated since the date of GALVANI's discovery. The possibilities of the insulating or semi-insulating sheath of the nerve, that is to say of the core model structure of the nerve, have been discovered from every point of view, but without any very direct attempt having been made to obtain experimental data which might place definite limits to these possibilities.

BORUTTAU and STRONG,¹ and perhaps HOORWEG (although his methods of illustrating his conception are open to objection), have realised the necessity entailed by the core model structure, namely, that its fracture must provide one with a current of injury. STRONG has presented a detailed description of his hypothesis, and BORUTTAU has illustrated a similar one by a working model,² but neither seem to have grasped the important corollary which their statements, if true, must entail, namely, that the injury current and its modification, under known conditions, may, or rather must, give valuable information as to the details of the core model structure.

The appreciation of this position necessitates a return to DU BOIS REYMOND's point of view and the establishment of the injury current as a phenomenon of primary importance.

NOTE ON THE PURELY PHYSICAL THEORY OF NERVE FUNCTION

The greatest objection to a physical theory of nerve function is the existence of recorded instances in which the transmission of the nervous impulse is proved to have taken place unaccompanied by a demonstrable physical phenomenon. Many of these instances are open to considerable suspicion, as are all physical experiments made by investigators satisfied with approximate and inexact methods and apparatus. Criticism of such recorded instances has already been undertaken by BORUTTAU, but there are certain broad principles affecting experiments of this kind which have not been as much insisted upon as is necessary.

1. Strong, *Journal of Physiology*, XXV, p. 427.

2. Borutttau, *Pflüger's Archiv*. LXIII, p. 154. 1896. Gelatine cylinders of K.Cl. solution surrounded by a mantle of .6 per cent. NaCl. solution. The model so formed shews a negativity of cross section to longitudinal surface.

In the first place, experimental modifications designed only to affect the physiologically interesting units of structure of the nerve (the axis cylinders) are also capable of producing great modifications in recorded electrical changes by altering the conditions of the complex wrappings which surround the axis cylinders. There is often reason to suppose that modifications in recorded electrical changes obtained after immersion of nerves in solutions of electrolytes, and even of non-electrolytes, are merely variations in the relative value of the outwardly demonstrable change. The real phenomenon being, perhaps, unaltered in value or else altered in a manner quite different from that in which its 'externally visible' moiety is affected.

The need of such criticism is obvious when variations are produced by such extreme means as the immersion of nerves in saturated salt solutions, but it is also applicable and almost invariably neglected in other less striking instances.

The relative value of the outwardly demonstrable change to the real phenomenon in the axis cylinder depends upon at least three factors—the physical characters (electrical conductivity, etc.) of the lymph, the nerve fibre sheath, and the axis cylinder. It is more than conceivable that a reagent which affects only the nerve sheath should, even if not in any way modifying the condition of the axis cylinder, produce an important variation in the outwardly accessible phenomenon; and there is no difficulty in extending such a proposition to include also the effect of reagents which alter the surrounding 'lymph' without affecting any part of the nerve fibre itself.

This proposition also holds good for experiments in which modifications are produced in the so-called 'excitability' by immersion of the nerve in solutions, etc., which may produce their apparent effects by altering the conditions under which the stimulating current arrives in or leaves the axis cylinder without affecting the axis cylinder itself in any manner.

Immersion in solutions, exposure to gases, variations of temperature, all alike may produce the major effect observed by altering the quantity of change which can make its way to the surface, or in the case of stimulation, by varying the amount of stimulation which reaches the axis cylinder from the surface of the nerve.

Nor is this the only consideration which places negative results under suspicions unless stringently examined, for some of the experiments which are presumed to present results of value in this connexion have involved other complications still more undesirable.

The ordinary diphasic record obtained by the usual means does not, it is acknowledged, present in either of its phases a correct, or even approximately correct, idea of the magnitude and duration of the electrical change traversing successive sections of the nerve. The record is the algebraical sum of two such real phases. In the generally chosen conditions of experiment, when the interval of nerve between the electrodes is a short one, and traversed by the nervous impulse in a time which is a fraction of the whole time occupied by the complete passage of the changes accompanying it past either point; under these circumstances large portions of the two opposite phases occur at the same time, and to a great extent are, therefore, eliminated from the record.

When statements are made as to the non-occurrence of portions of the anticipated electrical change, it is as well to immediately consider the possibilities of such elimination. Important as this is when only a single nervous impulse is presumed to have passed the two electrode points, it becomes of vital interest when a second has been despatched in rapid succession to the first, for then the record becomes the algebraical sum of four phases, and is unrecognizable for any useful purpose. It is an easy matter to demonstrate that such an algebraical sum may apparently present four phases, or three, or even two; and in fact may so closely imitate a diphasic record, the record resulting from the passage of a single nervous impulse, as to be mistaken for this. Such a record may then be, and probably has actually been, held out as a proof that the second nervous impulse did not in its passage give rise to a second travelling electrical change.

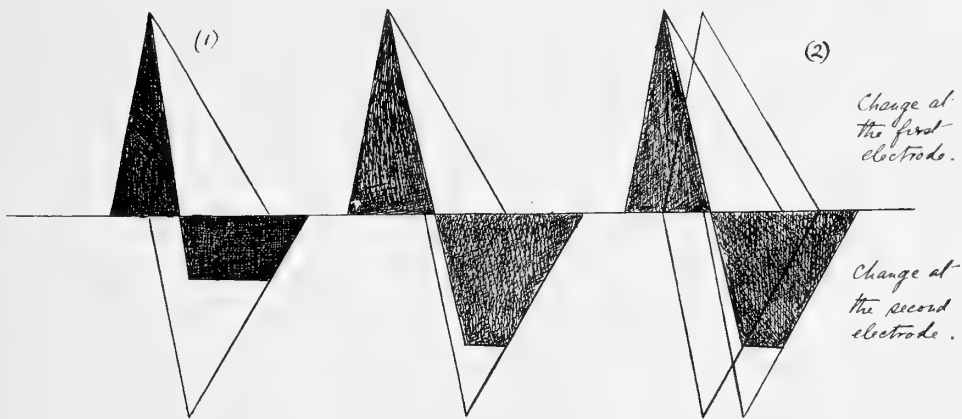


FIG. 1

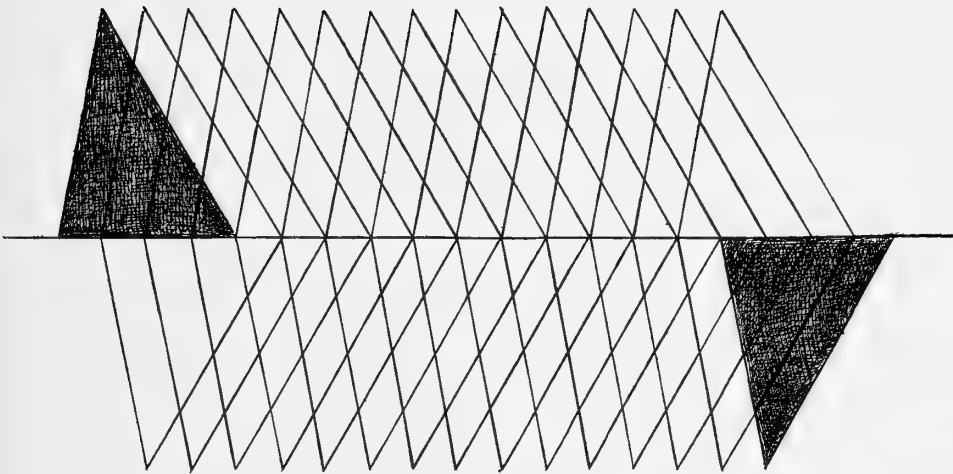


FIG. 2

Fig. 1. The three drawings represent—

(1) A diphasic record, the result of one nervous impulse passing two points separated by a time interval of '001".

A diphasic record. One nervous impulse. Two points '002" apart.

(2) A diphasic record. Two nervous impulses. Two points '001" apart.

Fig. 2. A diphasic record. Fifteen nervous impulses. Two points '001" apart.

In the accompanying figure the two darkly-shaded records (1 and 2) are two diphasic records of not very different character. The first is the outcome of *one change*, affecting two points in succession, at an interval of onset of '001 second, and lasting at each point for '004."

The second is the result of *two such changes* passing the same two points with an interval between them of '001."

It is obvious that in the second two out of the four phases actually occurring are entirely unrepresented in the record, since occurring at the same time and being equal and of opposite sign they are completely eliminated from it. *The actual record, although the result of two nervous impulses, let us say, passing at an interval of '001, is exactly the same as the record would be of one nervous impulse passing along the nerve if its passage was observed by placing electrodes upon two points separated by double the distance which divides the two points arbitrarily chosen.*

To emphasize the statement, it is interesting by an examination of fig. 2 to convince oneself that the passage of ten nervous impulses at the rate of one thousand per second, or of one thousand nervous impulses at the same rate, and even if each in succession produced at every point its appropriate electrical change might in a record give rise to a *diphasic* variation. In this extreme case the diphasic record could hardly be interpreted as due to the occurrence of only one electrical change at each point.

Such considerations hold good for every experimental record taken as the observed effect of two or more successive stimuli, and vitiate, in a very great number of cases, conclusions drawn in their neglect. When many successive stimuli are used, as in faradisation, and when imperfect recording instruments add further fallacies to the record, as in galvanometric observations, it is impossible to base any conclusion of even suggestive interest upon such records. It may even be reasonably said that such criticism applied to diphasic and multiplied diphasic records obtained by the observation of two points in the continuity of the nerve, may be transferred to records of 'negative variations,' taken from the longitudinal surface and cross section. Confidence is only implicitly placed in such records when it is imagined that electrical change only occurs at the point on the longitudinal surface, and that the real change is, therefore, monophasic. This is, however, far from being the truth, for even exactly-taken records of such single negative variations are triphasic: and no one can satisfactorily declare what the nature of the real phases occurring at either point was, which has resulted in this triphasic record.

THE CURRENT OF INJURY

The statements subsequently made are all based upon observations taken from experiments upon mammalian nerve, which offers several advantages for the purposes of this enquiry.

In the first place, mammalian nerve can be obtained in comparatively long stretches, and in the case of the phrenic or the vagus nerves in long stretches free from branches and, therefore, from undesirable accessory cross sections. The phrenic nerve, in addition, offers the advantage of containing only medullated fibres of uniform size and relative value of axis cylinder and myelin; it is, however, not easy to remove as neatly from its pleural covering as are nerves placed in a bed of loose areolar tissue, and on this account, although at first made use of, it has been for the present abandoned.

Certain mammalian nerves, such as the vagus of the dog, although for many reasons preferable, suffer from the disadvantage of exhibiting a relatively small difference of potential between the longitudinal surface and the cross section. It is in such cases naturally more difficult to form a correct opinion of the real distribution of potential upon the surface of the nerve.

This apparent disadvantage is, however, discounted by the comparatively small resistance of mammalian nerve in general, as contrasted with the relatively minute sciatic nerve of the frog. The resistance in the nerve being small, a slight potential difference gives rise to an appreciable current through the galvanometer, and compensation and determination of potential difference is in this manner rendered far more exact. The disadvantage has also in the present case been of no interest since an extremely sensitive galvanometer (Thompson pattern, Muirhead and Company) of 50,000 ohms. resistance has been used throughout the whole series of experiments.

The differences of potential measured in the case of the vagus nerve recently removed from the dog were rarely as great as $\cdot 010$ volt, averaging about $\cdot 007$ volt. In the case of the sciatic nerve of the dog and cat the potential differences obtained are much greater, and are greater than the potential difference ordinarily obtained from the sciatic nerve of the frog. The frequent examples given subsequently in this paper will be found fully to bear out this statement. Consequently, it is easy in the case of the mammalian sciatic nerve, dealing with a low resistance and a large difference of potential, to measure accurately the comparatively large currents which are found and to compensate the potential differences with ease. The results of a typical examination of a sciatic nerve are given in Experiment I.

In this case, as in all cases examined, certain irregularities will be noticed, showing a departure from the ideal case depicted by DU BOIS REYMOND's laws. It is convenient to assume that such irregularities are produced by accessory cross sections; it is not however believed that all, nor even the most important, irregularities are the outcome of such an accidental cause. It will be seen that experiments made upon carefully prepared and treated vagus nerves are not free from irregularities of an exactly similar kind, and in this connexion attention is drawn to the facts of Experiment (Tap Water, fig. W, see p. 279), in which the irregularities are demonstrated in a very striking manner by the use of a special expedient.

In this section the important details of experiments are given which may serve to demonstrate the conditions actually to be met with. No attempt is made to arrange them with reference to any hypothetical simplicity, it being considered that the examination of actual curves, experimentally obtained, is of greater value. It is necessary to exhibit the phenomenon in some detail, because such a demonstration of actual measurements has never been previously undertaken, and because great pains have been taken to correct the observations made from the consequences of an unavoidable source of error most frequently neglected.

This source of error is due to the alteration in the phenomenon taking place with lapse of time, but fortunately taking place, as will subsequently be seen, in a regular and definite manner. The change with lapse of time is not greater in mammalian nerve than in frogs; it is, however, in either case considerable, and as the data of the experiment given below show, the error, unless corrected, is always vitiating the conclusions drawn from observations. Comparisons between differences of potentials found between sets of points, such as are made when an attempt is made to study the distribution of potential, are valueless, unless the routine method of conduction of the experiment permits the comparison to be made between corrected values *inferred to exist at the same moment of time*.

EXPERIMENT A

VAGUS NERVE OF CAT

Piece of Nerve 6 centimetres long

The nerve was laid upon a dry ebonite scale forming a platform in the moist chamber. One non-polarizable electrode was placed in contact with a cross section and retained there throughout the experiment; the other non-polarizable electrode was first placed in contact with a point distant 1 centimetre from this, then upon a second point distant 2 centimetres, a third at 4 centimetres, a fourth at 5 centimetres distance, an observation being taken in each case of the potential difference between each point and the cross section. The experiment was continued by a return of the second electrode to point (4), then to point (3), then to point (2), then to point (1). Thus two observations were taken at each point, once going up and once going down the nerve, *and a definite interval of time was allowed for each observation*.

In the figures given below, and in the case of all other experiments recorded in this section, the arrows indicate the order in which the observations were taken :—

Distance of point from the cross section			Potential Difference between this point and the cross section		
			(a)		(b)
(1)	1 centimetre	...	·01904	↓ ↑	·01416 Daniell
(2)	2	„	·01696		·01624 „
(3)	3	„	·01696		·01688 „
(4)	4	„	·01672		·01688 „
(5)	5	„	·01520		·01520 „

If the two sets of observations (a) and (b) of this experiment are taken singly, it is obvious that a different idea is capable of being formed from each of them of the distribution of potential upon the longitudinal surface. The maximum of the curve representing this distribution is in case (a) obviously to be placed between points (2) and (3), point (3) being the mid-point of the piece of nerve examined. The maximum in case (b) is to be placed between points (3) and (4) and, therefore, on the other side of the mid-point. The reason for this difference between the two sets of observations is readily seen from the fall in the potential difference which has taken place at point (1) in the time necessary to take the whole set of observations, a fall of ·005 volt, that is of almost 25 per cent. of the initial value. The effect of this fall is not noticeable in the two observations at point (4) which rapidly followed upon one another; in fact, there is apparently a slight increase, due probably to the selection of a slightly different point upon the return visit. The fall has nevertheless been taking place all the time, and, provided that it is regular and that the observations have been taken at regular intervals, its effects can be eliminated by taking the average of such a set of observations; for corrected values are then obtained, which are almost accurately those actually existent at the moment of 'mean time' of the whole set of observations.

Taking this set of average values from this experiment, we have :—

Point (1)	·01665	Daniell
„ (2)	·01660	„
„ (3)	·01697	„
„ (4)	·01680	„
„ (5)	·01520	„

This set of figures provides a totally different idea of the distribution of potential. There is not a great difference between the whole set of values, the curve of distribution is, therefore, a comparatively flat-topped one; and its small maximum is at the mid-point of the nerve.

Necessary as is such a precaution when only five points are examined, it becomes doubly necessary when, for the sake of accuracy, more points are dealt with, and when a longer piece of nerve is taken. It has been found, with practice, possible to take twenty observations, two at each of ten points (travelling up and down in this way) in ten minutes. The success of the method and the value of the precaution are, in my opinion, both shown in the details of the following experiment:—

EXPERIMENT I

SCIATIC NERVE OF DOG

Piece of Nerve 8 centimetres long, clean cut at both ends

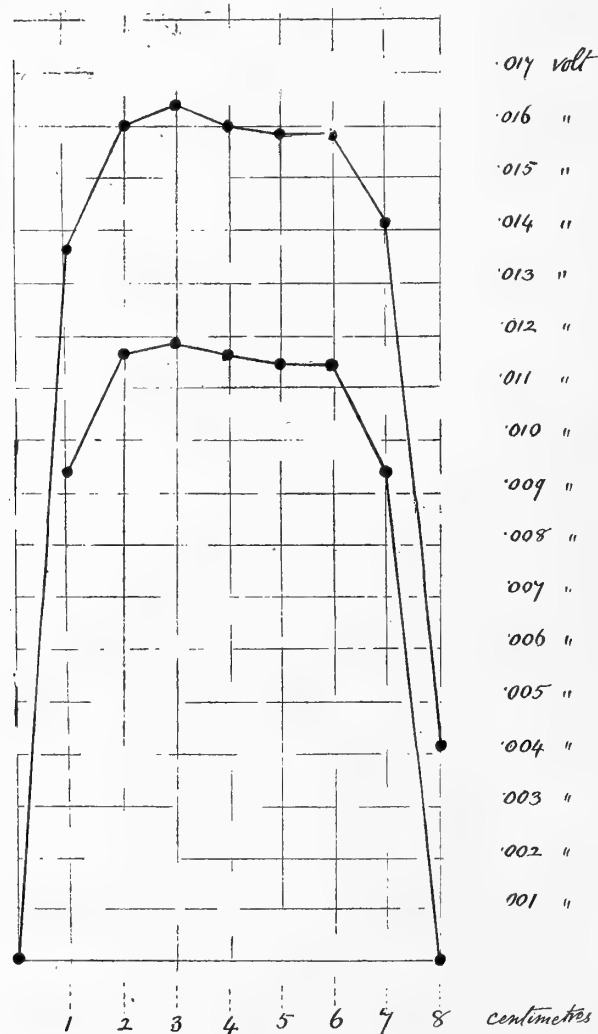


FIG. 3

Three double sets of observations were taken :—

- (1) The potential differences between points upon the longitudinal surface and the upper cross section.
- (2) The potential differences between the same points and the lower cross section.
- (3) A repetition of (1).

Each double set of observations occupied ten minutes. The total time spent over the three sets was thirty minutes; no interval was allowed between one set and another, and the taking of every observation was accurately timed.

For the sake of simplicity, the description of the geometrical position of each point is always the same throughout the whole set of observations. Whether the potential difference is being taken between a point and the upper or the lower cross section, the distance of the point from the upper cross section provides it with its name. Thus point (1) is always a point distant 1 centimetre from the upper cross section, etc.

The order in which the figures are given and the arrows drawn alongside of them fully indicate the order in which the observations were made.

The difference between the two cross sections was only once measured, and the upper cross section was found to be negative to the lower by $\cdot 00422$ volt, the relative time at which this measurement was taken will be seen from the list of observations in which it is recorded. *It was measured at the mean time* of the whole set of observations. Point (8) is marked with an asterisk to emphasize the measurement taken between the two cross sections, and the same plan has been followed in all the other experiments of this section.

There was no potential difference between the electrodes, and there never is in the experiments given any reason to apply a correction for such a difference. Differences between electrodes are usually the result of a slight difference in the concentration of the zinc sulphate solution in the two tubes, the result of pouring zinc sulphate solution into imperfectly dried tubes. This error has been completely avoided by the use of very wide U tubes, having a bore of 1.5 centimetres, so that they contain a large volume of the solution, and by cleaning them always the night previous to the experiment, and using them dry in the morning without resorting to any further cleaning process.

The nerve was, as in the last experiment and all succeeding ones, laid on an ebonite scale in a large moist chamber (25 centimetres long and 25 centimetres broad by 10 centimetres deep), the electrodes were freely moveable upon ebonite runners placed in this moist chamber, parallel to and one on either side of the scale on which the nerve was placed. The greatest care was taken to secure perfect insulation of every piece of apparatus made use of.

A			B			C		
Potential Differences taken from the Upper Cross Section			Potential Differences taken from the Lower Cross Section			Potential Differences taken from the Upper Cross Section		
(1)	·01491	·01439	·00950	·00898	·01307	·01214		
(2)	·01755	·01737	·01209	·01130	·01492	·01426		
(3)	·01795	·01803	·01228	·01156	·01555	·01452		
(4)	·01795	·01756	·01188	·01135	·01465	·01412		
(5)	·01769	·01742	·01162	·01120	·01412	·01399		
(6)	·01769	·01756	·01148	·01135	·01399	·01399		
(7)	·01565	·01531	·00924	·00924	·01294	·01294		
(8)*	·00422	·00422						

BELOW ARE GIVEN THE AVERAGE VALUES TAKEN FROM A, B, AND C
RESPECTIVELY

A Potential Differences to upper		B Potential Differences to lower		C Potential Differences to upper	
(1)	·01465		·00924		·01260
(2)	·01746		·01169		·01459
(3)	<u>·01799</u>		<u>·01192</u>		<u>·01503</u>
(4)	·01775		·01161		·01438
(5)	·01755		·01141		·01405
(6)	·01762		·01141		·01399
(7)	·01548		·00924		·01294
(8)*	·00422				

* Point (8) represents the second lower cross section.

BELOW IS GIVEN AN AVERAGE OF A AND C FOR COMPARISON WITH B

A and C Potential Differences to upper			
(1)	·01362	(5)	·01580
(2)	·01602	(6)	·01580
(3)	<u>·01651</u>	(7)	·01420
(4)	·01606	(8)	—

We have in the figures of this experiment the means of contrasting the distribution of potential on the nerve at the same moment of time as found by comparison with the upper and the lower cross section, and that also at a moment of time when the potential difference between these two cross sections was observed (·0042 volt).

	Potential Differences to upper	Potential Differences to lower	Arithmetical Differences
(1)	·01362	·00924	·00438
(2)	·01602	·01169	·00433
(3)	·01651	·01192	·00459
(4)	·01606	·01161	·00445
(5)	·01580	·01141	·00449
(6)	·01580	·01141	·00449
(7)	·11420	·00924	·00496

The two curves drawn from these numbers are given in fig. 3, and it is obvious, both in the figure and in the numbers given above, that the curves are parallel and reproduce at a different level every minute phase. Further, the difference of level between the two curves ·004 volt (approx.) is almost exactly the difference found at that moment of time between the two cross sections. If the mill of observations out of which this similarity has been evolved is considered, it will serve to establish confidence in the meaning of every curve taken in this routine fashion. The difficulties to contend with are found not only in the fall of level with lapse of time, but also, as more curves drawn from the numbers given above would show, an alteration in the form of the top of the curve. The alteration in form is, however, as regular as the fall of level, and, like it, may have a meaning which will repay investigation; it is most clearly appreciable in the cases of nerves removed from animals dead some hours (when the surface of the nerve is in a peculiar condition, which is subsequently discussed); in the case of freshly removed nerves from recently killed animals it is, as in this case, only slight.

SUMMARY

In general the points which one would like to emphasize in the data of this experiment are:—

(a) That, as DU BOIS REYMOND noted, the 'equatorial' point, or highest point of the curve, does not correspond with the geometrical centre of the nerve.

(b) That there is a difference between the two cross sections; but that in this case, although by no means in all cases, the highest maximum of the curve is nearer to the more negative cross section.

(c) That the fact of changing the point of reference from one cross section to another does not, in the slightest degree, alter the relative value of points upon the longitudinal surface to one another.

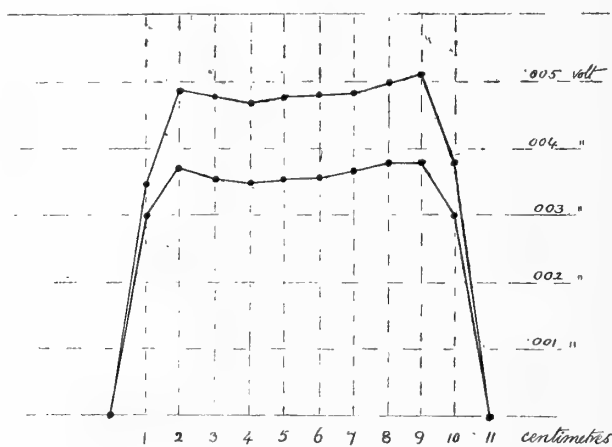
(d) That the lapse of time does reveal a definite but small alteration in the relative value of points upon the longitudinal surface. In this case the alteration is such as to make the potential curve a more obviously asymmetrical one.

The experiments upon vagus nerves which follow are given for the present without comment as examples, carefully worked out, of the distribution of potential upon excised nerve.

EXPERIMENT II

VAGUS NERVE OF DOG

Piece of Nerve 11 centimetres long. Ligatured and cut at both ends



II

Points upon the longitudinal surface distant from the other end	Potential Differences measured from the upper injury		Average Value (correction for alteration with time)
1 centimetre ...	·00352 D.	·00328 D.	34.0×10^{-4} D.
2 " ...	·00508	·00446	47.7
3 " ...	·00500	·00432	46.6
4 " ...	·00492	·00432	46.2
5 " ...	·00508	·00444	47.6
6 " ...	·00500	·00452	47.6
7 " ...	·00508	·00452	48.0
8 " ...	·00508	·00480	49.4
9 " ...	·00520	·00504	51.2
10 " ...	·00372	·00372	37.2

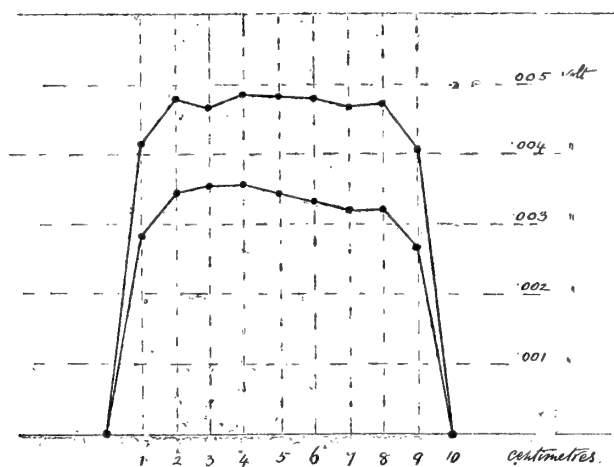
After an interval of thirty-five minutes, a second double set of observations was taken. The interval between the mean times was forty-five minutes.

Points upon the longitudinal surface distant from the other end	Potential Differences measured from the upper injury		Average Value (correction for alteration with time)
1 centimetre ...	·00272 D.	·00296 D.	28.4×10^{-4} D.
2 " ...	·00348	·00360	35.2
3 " ...	·00348	·00340	34.4
4 " ...	·00340	·00340	34.0
5 " ...	·00348	·00344	34.6
6 " ...	·00348	·00352	35.0
7 " ...	·00348	·00368	35.8
8 " ...	·00372	·00376	37.4
9 " ...	·00372	·00372	37.2
10 " ...	·00280	·00286	28.3

EXPERIMENT III

VAGUS NERVE OF DOG

Piece of Nerve, 10 centimetres long, both ends cut



III

Point on the longitudinal surface distant from the upper end of the nerve	Potential Differences measured from the Upper Cross Section		Average Value (Correction for alteration with lapse of time)
1 centimetre ...	·00448 D.	·00400 D.	42.4×10^{-4} D.
2 " ...	·00520	·00472	49.6
3 " ...	·00500	·00472	48.6
4 " ...	·00544	·00472	50.8
5 " ...	·00544	·00456	50.0
6 " ...	·00544	·00451	49.7
7 " ...	·00520	·00440	48.0
8 " ...	·00520	·00464	49.2
9 " ...	·00416	·00424	42.0

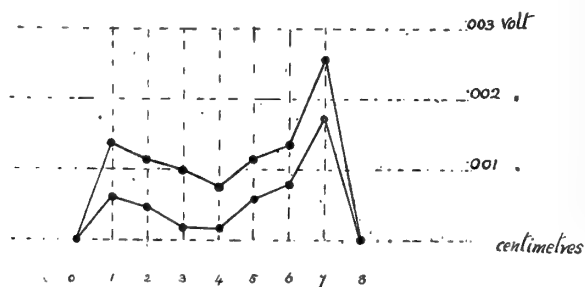
A second set of observations, taken after an interval of thirty-five minutes, *i.e.*, with an interval between the mean times of the two sets of forty-five minutes.

Point on the longitudinal surface distant from the upper end of the nerve			Potential Differences measured from the Upper Cross Section		Average Value (Correction for alteration with lapse of time)
1 centimetre	...		·00328 D.	·00248	28.8×10^{-4} D.
2	„	...	·00392	·00320	35.6
3	„	...	·00406	·00320	36.3
4	„	...	·00408	·00320	36.4
5	„	...	·00392	·00317	35.4
6	„	...	·00368	·00317	34.2
7	„	...	·00349	·00296	32.2
8	„	...	·00349	·00296	32.2
9	„	...	·00272	·00272	27.2

EXPERIMENT IV

VAGUS NERVE OF DOG

Piece of Nerve, 8 centimetres long, removed seven-and-a-half hours after death. Distance measured from upper end. Point (1) = 1 centimetre from upper end. Point (2) = etc.



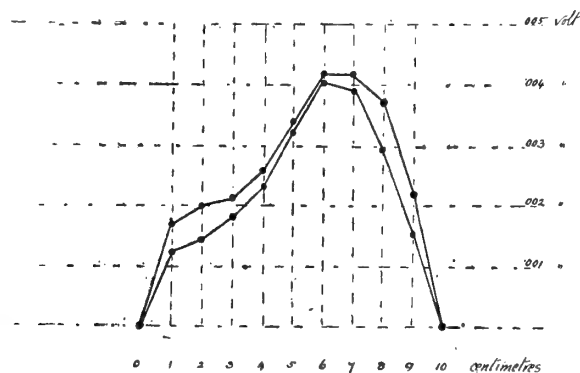
DIFFERENCES OF POTENTIAL

To Upper Cross Section			Average	To Lower Cross Section			Average
(1)	.00128	.00140	.00134	.00056	.00064		.00060
(2)	.00104	.00128	.00116	.00024	.00064		.00044
(3)	.00096	.00104	.00100	.00008	.00024		.00016
(4)	.00072	.00080	.00076	.00008	.00024		.00016
(5)	.00112	.00120	.00116	.00056	.00064		.00058
(6)	.00128	.00144	.00136	.00064	.00104		.00082
(7)	.00264	.00264	.00264	.00224	.00224		.00224

EXPERIMENT V

VAGUS NERVE OF DOG

Piece of Nerve, 11 centimetres long. Cross section, upper end. Ligature below. Distances all measured from the upper cross section. Nerve removed two-and-a-half hours after the death of the animal.



DIFFERENCES OF POTENTIAL

To Upper Cross Section			Average	To Lower Cross Section			Average
(1.5)	·00140	·00216	·00178	·00124	·00124	·00124	·00124
(2.5)	·00136	·00260	·00198	·00140	·00156	·00148	·00148
(3.5)	·00160	·00260	·00210	·00164	·00220	·00192	·00192
(4.5)	·00216	·00312	·00264	·00180	·00300	·00240	·00240
(5.5)	·00292	·00404	·00348	·00308	·00352	·00330	·00330
(6.5)	·00380	·00464	·00422	·00424	·00400	·00412	·00412
(7.5)	·00380	·00464	·00422	·00412	·00372	·00392	·00392
(8.5)	·00312	·00360	·00386	·00288	·00308	·00298	·00298
(9.5)	·00224	·00224	·00224	·00152	·00164	·00158	·00158

EXPERIMENT VI

VAGUS NERVE OF DOG

Piece of right vagus, 9.5 centimetres long, removed immediately after death. Clean cut cross section at either end. No ligatures. Distances measured from the upper cross section.



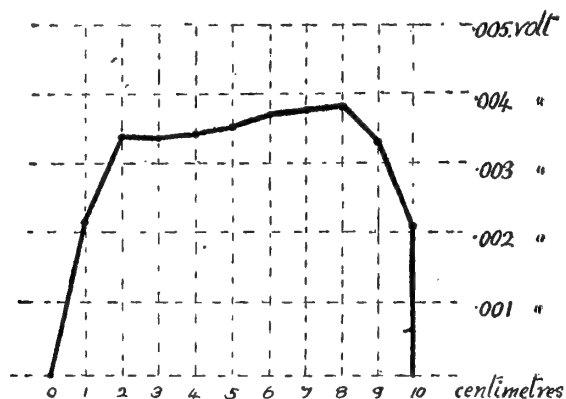
Potential Differences from the Upper Cross Section			Average
Point (1)	.00360	.00312	.00336
" (2)	.00432	.00372	.00402
" (3)	.00452	.00400	.00426
" (4)	.00460	.00428	<u>.00444</u>
" (5)	.00460	.00400	.00430
" (6)	.00460	.00432	<u>.00446</u>
" (7)	.00460	.00432	<u>.00446</u>
" (8)	.00460	.00424	.00442
" (9)	.00288	.00307	.00297
" (9.5)*	0	0	0

Points marked thus * are in each case the second cross section.

EXPERIMENT VII

VAGUS NERVE OF DOG

Piece of Nerve, 10 centimetres long, removed immediately after death. Both ends ligatured and cut close to the ligature. Distances measured from the upper cross section.



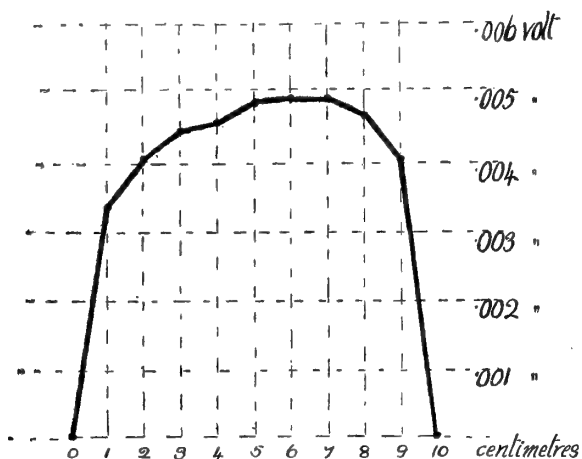
Potential Differences from the Upper Cross Section			Average
Point (1)	·00224	·00216	·00220
„ (2)	·00344	·00344	·00344
„ (3)	·00344	·00340	·00342
„ (4)	·00360	·00340	·00350
„ (5)	·00360	·00344	·00352
„ (6)	·00364	·00368	·00366
„ (7)	·00372	·00368	·00370
„ (8)	·00384	·00368	<u>·00376</u>
„ (9)	·00328	·00328	·00328
„ (10)*	·00212	·00212	·00212

Points marked thus * are in each case the second cross section.

EXPERIMENT VIII

VAGUS NERVE OF DOG

Piece of Nerve, 10 centimetres long, removed immediately after death. Both ends cut. No ligatures. Distances measured from the upper cross section.



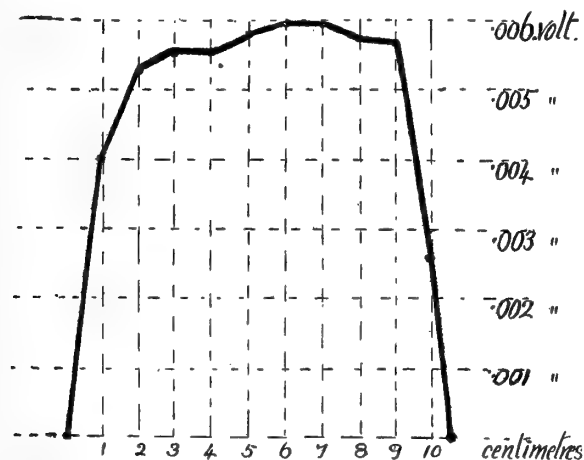
Potential Differences from Upper Cross Section			Average
Point (1)	·00360	·00296	·00328
„ (2)	·00424	·00368	·00396
„ (3)	·00456	·00424	·00440
„ (4)	·00488	·00412	·00449
„ (5)	·00520	·00432	·00476
„ (6)	·00504	·00456	<u>·00480</u>
„ (7)	·00496	·00472	<u>·00484</u>
„ (8)	·00496	·00424	·00460
„ (9)	·00412	·00384	·00397
„ (10)*	·00016*	·00016	·00016

Points marked thus * are in each case the second cross section.

EXPERIMENT IX

VAGUE NERVE OF DOG

Piece of Nerve, 10.5 centimetres long, removed one hour after death. Nerve ligatured and cut at both ends. Distances measured from the lower injury.



Potential Differences Measured from the Lower Cross Section			Average
Point (1)	·00320	·00468	·00394
„ (2)	·00468	·00584	·00526
„ (3)	·00520	·00584	·00552
„ (4)	·00520	·00584	·00552
„ (5)	·00552	·00604	·00578
„ (6)	·00572	·00600	<u>·00586</u>
„ (7)	·00572	·00600	<u>·00586</u>
„ (8)	·00556	·00584	·00570
„ (9)	·00552	·00576	·00564
„ (10)	·00256	·00256	·00256
„ (10.5)*	not observed		

Points marked thus * are in each case the second cross section.

MODIFICATION OF THE DISTRIBUTION OF POTENTIAL BY THE 'EXTERNAL ARC'

CHANGES IN THE DISTRIBUTION OF POTENTIAL PRODUCED BY THE CONDITIONS OF AN EXPERIMENT

A necessary step in the process of examination of the nerve is the application to it of two electrodes and a connecting wire path. The whole arrangement with the included galvanometer may conveniently be termed an observation circuit, or, simpler still, an 'external arc.' It is a well-known fact that the distribution of potential on the surface of the nerve, as on any other conductor, is unaffected by the presence of such an external arc when the current found traversing it has been accurately compensated. It is also a well-established, but by no means so generally known, fact that the presence of such an external arc profoundly modifies the distribution of potential upon the surface and in the interior of the intervening piece of nerve, when the current traversing the arc is left uncompensated.

The first part of this statement, namely that the external arc with compensated current is not a disturbing factor, is of obvious importance to the actuality of the results obtained by this method of examination, and it was shown by HELMHOLTZ¹ to be mathematically true. The value of the error due to an absence of compensation was also calculated by him, and that, too, with a special reference to the case of animal tissues and the centres of electromotive activity, assumed by DU BOIS REYMOND to be imbedded in them; the calculation showed that the distribution of potential was modified in a very precise manner. The effect of placing an external arc upon such a conductor, and the consequent derivation of a current through the arc, was shown so to alter the distribution of potential in the conductor as if the conductor formed a portion of the circuit through which the current through the arc was flowing. Thus in the case of a nerve, the current flowing in the galvanometer circuit from longitudinal surface to cross section exactly modifies the pre-existing distribution of potential in the nerve, as if it also flowed in the nerve from cross section to longitudinal surface.

The truth of the calculations was also demonstrated in experiments performed upon conductors in which sources of EMF were placed; but the actual demonstration of the quantitative influence of this condition in experiments upon nerve has never been undertaken. The presence of the condition has, indeed, been noticed in physiological experiments, as, for example by VON FLEISCHL,² in observations

1. For a detailed account of Helmholtz's postulates see Adolf Fick, *Die Medizinische Physik*, 1858, p. 354; Morgan, *Electrophysiology, etc.*, p. 265. New York, 1868.

2. E. v. Fleischl, *Electrotonus, etc., Sitzungsber d. Wien, Acad.*, LXXVIII, Abth. 3.

upon electrotonic currents (when HERMANN¹ identified it as being due to this cause, confirming his statements by experiments upon the core-model), nevertheless, it has not been directly examined.

The experiments given below may serve to supply such a deficiency, although not made with this intention, since they were undertaken in ignorance of the general principle enunciated by HELMHOLTZ, and with a view to experimentally investigate the disturbance produced by the placing of an 'external arc.' The examples, which are quoted, all exhibit special instances of the manner in which the pre-existing difference of potential is affected, *e.g.* :—

- (a) The creation of differences of potential where none existed previously.
- (b) The diminution, elimination, or reversal of pre-existing differences of potential.

The agreement of the results actually found with those anticipated by a law unknown to the investigator is evidence of the general exactness of the measurements taken in this research, and also, and this is of importance, to the correctness of certain assumptions made in dealing with the measurements of resistance.

EXPERIMENTS

In each experiment the nerve, having been removed from the animal, was placed upon four nonpolarizable electrodes—A, B, C, D; the cross section being always placed upon electrode A, and the other end of the nerve, extending beyond electrode D, was suspended from the wall of the moist chamber by a silk thread.

The wires connected to the four electrodes—A, B, C, D—were fitted into brass plugs, which could readily be inserted and removed from positions in circuits arranged for the measurement of potential differences or resistances, or could be placed into adjoining holes in an insulated piece of brass. In this way it was possible to measure the resistance, take the potential difference, or complete the 'arc' joining the electrodes simply, or through an inserted resistance.

The length of the nerve and of its various sections, as divided by the position of the electrodes, was carefully measured.

The resistances of the nerve and of its various sections were also measured. Each value given for a resistance being the mean of two measurements taken with the nerve placed in the two positions possible in the limb of the Wheatstone bridge. This precaution was taken to avoid the error due to the presence of differences of potential.

The resistances between the electrodes themselves were measured, for each pair, before and after the experiments. The values obtained for these have in each case been subtracted from the values obtained of the resistances of pieces of nerve inclusive of electrodes.

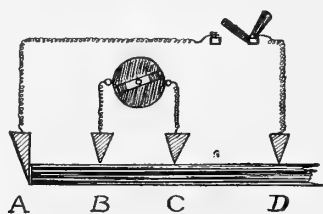
1. Hermann, *Pflüger's Archiv*, XX, 1879; also H. Weber, *Borchardts Journ. of Math.*, LXXVI, p. 13.

The potential differences between the electrodes were measured before and after the experiments; no experiments are recorded in which any such were found, and, as has been previously stated, this latterly has never been the case.

A complete set of such measurements having been taken, the experiment was performed. Electrodes A and D were connected together through the plug key, so as to allow the injury current to traverse the arc AD. When this was done it was always found that the potential difference between the intermediate points, B and C, was greatly altered. The alteration remaining constant as long as AD was closed, and disappearing immediately when this arc was broken.

The performance of the experiment was followed by the making of a simple calculation from the data then obtained, by which it was sought to reveal the relation which might exist between the alteration in the potential difference between points B and C and the resistance between these points in the path of a current traversing the arc DA and the nerve AD as a complete circuit.

The basis of this calculation was the value of the resistance between points B and C, and also the resistance in the whole circuit inclusive of the nerve AD.



It is necessary, before proceeding to the details of the actual experiments, to consider the comparative value and meaning of the measured resistances of the pieces of nerve—AD and BC.

There is no difficulty in determining the required resistance of the whole circuit, and thus of the piece of nerve AD to the terminal points of which the observation arc is applied. For the resistance in this case is necessarily measured by the resistance to a current entering and leaving the nerve at the points A and D, that is at the points and traversing the same path as the current determined by the position of the arc.

The measurement of resistance of the short piece of nerve BC is different, since the current used to measure this resistance must enter and leave the nerve at points B and C, and encounter a 'transverse' resistance in so doing which is not encountered by a current passing, as in the assumed conditions of the experiment, from A to D and through points B and C, situated within the longitudinal resistance.

This objection invalidates all conclusions made as to the resistance of nerve measured between two points upon its longitudinal surface, just as the measurement of the resistance between two points upon the surface of an insulated, or rather badly insulated cable, is of no value, when one is seeking information as to the conductivity which the cable offers to currents traversing the same section of cable but passing in the interior of the cable from and to points beyond the terminals of the small stretch considered.

There can be no question but that the direct measurement of the resistance of short pieces of nerve is rendered in this way fallacious, it seems possible, however, that a study of the extent of the fallacy may be of interest. Direct experimental evidence justifying the rejection of such measurements may be taken from the observation of differences in the resistance per centimetre of long and short stretches of nerve. For if a piece of nerve is laid upon several electrodes, and the resistance of the whole piece of nerve and of its parts separately measured, it is always the case that the directly measured total resistance is less than the same resistance computed by the addition of the resistances of the several parts; a fact undoubtedly due to the repeated inclusion of the transverse resistance in the measurements of the shorter stretches. The finer the calibre of the nerve and the longer the distance between each pair of electrodes the less true this statement becomes, obviously because there is then a closer approach to 'infinity' of resistance of each small piece of nerve. The fact is, however, readily demonstrated upon the sciatic nerve of the frog, and disappears in such a nerve upon 'cooking,' in company with the excess transverse resistance. The fact is very apparent in large calibred mammalian nerve.

PRELIMINARY EXPERIMENT. SCIATIC NERVE OF DOG

The nerve was laid upon five electrodes—A, B, C, D, E

The resistances by sections	...	AB	6,150 ohms.
		BC	7,890 „
		CD	10,450 „
		DE	11,450 „
			<hr/>
Addition	...		35,940 ohms.
Resistance of AE, directly measured	...		24,000 „
			<hr/>
Difference	...		11,940 ohms.

i.e., an excess of 48 per cent. above the direct estimation.

The facts from similar experiments upon vagus nerves (dogs) are tabulated below to show in the briefest possible manner the magnitude of the error introduced into the examination of the resistance of short stretches of nerve.

TABLE OF PRELIMINARY EXPERIMENTS

RESISTANCE OF THE VAGUS NERVE OF THE DOG FROM SEVEN SEPARATE EXPERIMENTS

Number of Experiment	Length of Nerve in Centimetres	Number of Sections	RESISTANCE IN OHMS		EXCESS	
			Direct Measurement	Summation of Resistance of Parts	In Ohms	Per Cent.
I	8	5	100,952	128,720	27,768	27.5
II	8.3	5	121,500	149,500	28,000	29.1
III	7.8	4	88,500	105,950	17,450	19.7
IV	7.3	4	91,000	111,000	20,000	22.0
V	6.2	4	70,900	94,780	23,880	33.6
VI	5.3	3	65,200	82,400	17,200	26.3
VII	3.8	3	50,200	79,200	29,200	36.6

A consideration of such evidence and of the probabilities of the case examined by themselves make the avoidance of values obtained by direct measurement for the resistance of short stretches of nerve a matter of necessity. It seems clear that the resistance per centimetre obtained from the measurement of the longest stretch of nerve available, provided, if possible, with two cross sections, is a measurement as free as possible from errors due to transverse resistance and polarization phenomena, and gives the best determination of the gross longitudinal resistance of the nerve.

In the following experiments, therefore, all resistances have been directly determined, but the directly determined resistances of the shorter stretches of nerve are not used for purposes of calculation. The nerves were laid upon an ebonite ruled scale during the measurement of resistances and the distances of the electrodes from one another were read upon the scale and noted, from these lengths the resistances of the shorter stretches of nerve have been calculated by use of the standard resistance per centimetre obtained from the whole length of nerve. Both values are, however, given to show the magnitude of the error thus avoided, and also to serve as material for the purposes of any criticism directed against this method of procedure.

EXPERIMENT A

VAGUS NERVE OF CAT

Nerve laid upon four electrodes—A, B, C, D

Length of AD (piece of nerve between electrodes A and D)	...	4.3 cms.	
„ BC	...	1.8	„
„ AB (distance of B from the cross-section)	...	1.3	„
Resistance of AD directly determined	...	128,000 ohms	(1)
„ BC	...	63,000	„ (2)
„ a pair of electrodes	...	7,000	„ (3)
„ circuit AD, including electrodes	...	135,000	„
Calculated resistance of BC from the resistance per cm. obtained from (1) and from the length BC	{ 54,000 „		
Potential difference between A and D...	...	0.00712 Daniell	
„ „ „ B and C...	...	0	„

These data having been collected, the experiment was performed :—

- (1) Electrode A was permanently connected to electrode D.

The potential difference between points B and C was re-examined—B, the point nearer to the cross section, was found positive to C, the more distant point, by 0.0028 Daniell.

The experiment was repeated rapidly several times, in each case B and C were equipotential when A was not connected to D, when this connection was made, B was positive to C, as given.

- (2) Electrode A was connected to D through 100,000 ohms. resistance—B positive to C again, but by 0.00168 Daniell.

- (3) Electrode A was connected to D by 150,000 ohms resistance—B positive to C, 0.00128 Daniell.

- (4) Electrode A connected to D through 200,000 ohms—B positive to C, 0.00108 Daniell.

Finally, the connexion between A and D being broken, B was found as formerly equipotential to C.

The potential difference between A and D, examined at the end of this experiment—A was negative to D, 0.00648 volt.

EXPERIMENT B

VAGUS NERVE OF CAT

Nerve laid upon four electrodes—A, B, C, D. Cross section at A.

Length of AD	...	6.2 cms.	
„ BC	...	2.6	„
„ AB	...	0.8	„
Resistance of AD	...	204,400 ohms	(1)
„ BC	...	104,200	„
„ Pair of electrodes	...	6,000	„
Total resistance in circuit AD (nerve and electrodes)	...	210,400 ohms.	
Calculated resistance of BC obtained from its length and the resistance per cm. from (1)	...	85,800	„

EXPERIMENT B—*continued*

Potential difference between AD (A -)	·00840 Daniell.
" " " BC (B -)	·00068 "
(a) Electrode A connected up to electrode D, then it was found that B was positive to C	·00252 "
(b) Electrode A connected to D through a resistance of 100,000 ohms, then B was positive to C	·00144 "
(c) Connexion between A and D broken, then B was negative to C	·00068 "

EXPERIMENT C

VAGUS NERVE OF CAT

Nerve laid upon four electrodes—A, B, C, D. Cross section at A.

Length of Nerve AD	3.9 centimetres	(1)
" BC	0.7 "	(2)
" AB	0.7 "	
Resistance of AD	102,200 ohms.	(3)
" Electrodes... ..	6,000 "	
∴ of AD + electrodes	108,200 "	
Resistance of BC	28,460 "	
Calculated resistance of BC (from 3, 2, 1)	18,300 ohms.	
Potential difference between AD (A -)	·00752 Daniell.	
" " BC (B -)	·00144 "	
A connected to D. B remained negative to C	·00012 "	

EXPERIMENT D

SCIATIC NERVE OF CAT

Nerve was laid upon four electrodes—A, B, C, D. Cross section at A.

Length of nerve AD	3.4 cms.	
" BC	0.7 "	
" AB	0.7 "	
Resistance of AD	18,500 ohms.	
" Electrodes	6,000 "	
" AD and electrodes	24,500 "	
" BC	10,870 "	
Calculated resistance of BC	$\frac{18,500}{3.4} \times .7 = 3,800$ ohms.	
Potential Difference between AD (A -)	·00960 Daniell.	
" " BC (B -)	·00224 "	
(a) Electrode A having then been jointed to electrode D, the potential difference between BC (B -)	·00076 "	

If, in these experiments HELMHOLTZ's general principle is applied and examined by means of the data given, it will be seen that it explains all the results obtained.

If it is true that the moment a current is derived through the arc DA that then the distribution of potential in the nerve is precisely altered as if the same

current passed from A to D along the nerve, it must follow that the difference of potential between any two points, whether upon the wire path or the nerve, are affected in a manner which can readily be calculated.

The newly created difference of potential between points B and C upon the nerve should be equal to

$$\frac{\text{Resistance between B and C}}{\text{Total resistance in circuit}} \times \text{Potential difference between AD}$$

Where a pre-existing difference of potential is found between B and C, the old and the new should algebraically sum. These simple calculations, made for the four experiments given, are embodied in the following table :—

DATA

Resistance of BC	Total Resistance	Potential Differences between Points AD	Potential Differences pre-existing between Points BC
Experiment A. (a) 54,000	135,000	·00712 Daniell.	0
(b) 54,000	235,000	„	0
(c) „	285,000	„	0
(d) „	335,000	„	0
Experiment B. (a) 85,800	210,400	·00840 Daniell.	·00068 Daniell.
(b) „	310,400	„	„
Experiment C. (a) 18,300	108,200	·00752	·00144 „
Experiment D. (a) 3,800	24,500	·00960	·00224 „

VALUE FOR SUBSEQUENT POTENTIAL DIFFERENCES BETWEEN POINTS B AND C.

	Calculated	Found
A. (a)	·00284	·00280
(b)	·00163	·00168
(c)	·00133	·00128
(d)	·00114	·00108
B. (a)	·00274	·00252
(b)	·00164	·00144
C. (a)	— ·00017	— ·00012
D. (a)	— ·00076	— ·00076

Adding together the figures from the four experiments obtained by the application of the general principle to the data collected.

$$\begin{array}{rcl}
 \text{Calculated} & 1225 \times 10^{-5} & \text{Daniell.} \\
 \text{Found} & 1168 \times 10^{-5} & \text{,,} \\
 \hline
 \text{Total Difference} & 57 \times 10^{-5} & \text{Daniell.}
 \end{array}$$

or a difference of 4.6 per cent.

The experiments have naturally been selected from others providing similar evidence, and have been chosen on account of the exactness of the agreement between value calculated and found.

Nor is this unfair as practice was obtained in performing the series of measurements required: for each experiment increased the rapidity with which they were taken, and led to important modification in the convenient arrangement of the necessary apparatus. The experiments were also made unsupported by the knowledge that their results must, if correct, be of the nature given.

The next and last experiment recorded in this section affords not only a confirmation of the results previously given, but also illustrates a new point. From its data it may be seen that HELMHOLTZ's general principle is not only true of the injury current but also of 'longitudinal currents.' In either case the institution of the outer observation circuit modifies the distribution of potential upon the surface and in the interior of the nerve and in the same way. The current which passes from point (1) to point (2) in the outer wire path also traverses as a new phenomenon the nerve from point (2) to point (1).

EXPERIMENT E

SCIATIC NERVE OF CAT

Nerve on electrodes A, B, C, D. Cross section at A.

Length of AD ... 4 centimetres.

„ BC ... 1.6 „

„ AB ... 0.7 „

Directly measured resistance of AD ... 13,000 ohms.

„ „ BC ... 10,700 „

„ „ Electrodes ... 6,000 „

Resistance of BC calculated = $13,000 \times \frac{1.6}{4} = 5,200$ ohms.

Potential difference between AD (A—) 0.01450 Daniell.

„ „ BC (B—) 0.00448 Daniell.

EXPERIMENT PERFORMED

(a) Electrode A connected to D through a closed key.

Potential difference between BC now (B -) .00064 Daniell.

Key connecting A to D opened.

Potential difference between BC now (B -) .00448 „

(b) Electrode A connected to D through a resistance of 100,000 ohms.

Potential difference between BC now (B -) .00390 Daniell.

Connexion between A and D removed.

Potential difference between BC now (B -) .00448 „

(c) *Experiment of new type.*

Electrodes B and C were connected through a key.

When this key was closed, the potential difference between points A and D was measured and found (A -)01305 Daniell.

When this key was opened, the potential difference between points A and D was measured and found (A -)01450 Daniell.

Treating as before the data from this experiment.

In case (a).—The potential difference between B and C due to the closure of the circuit forming AD,

$$= .0145 \times \frac{5,200}{19,000} = .00397 \text{ (B +) Daniell.}$$

The pre-existing potential difference between these points00448 (B -) Daniell.

The algebraical sum of these values00051 (B -) „
and this is the calculated value.

The value actually found00064 (B -) „

In case (b)

The potential difference between BC due to closure of the circuit AD.

$$\begin{aligned} &= .0145 \times \frac{5,200}{119,000} \text{ Daniell (B +)} \\ &= .00062 \text{ Daniell (B +)} \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (1) \end{aligned}$$

But B was originally .00448 (B -) (2)

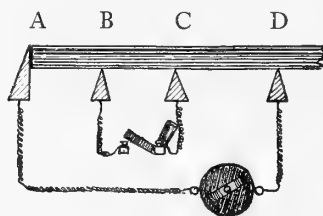
The algebraical sum of these values = .00386 Daniell (B -), and this calculated value closely agrees with the value found which was .00390 Daniell (B -)

	Value calculated	Value found
In case (a)	.00051 (B -)	.00064 (B -)
(b)	.00386 (B -)	.00390 (B -)

Case (c) requires to be considered by itself.

In considering the data from case c (Experiment E), the circuit formed by the closure of electrodes B and C, and by the intervening stretch of nerve BC is alone

considered. The remaining pieces of nerve AB and CD are treated, for the purposes of calculation, as if they were a continuation of the terminal conductors of the galvanometer and compensator circuit up to the points B and C.



According to HELMHOLTZ's principle the closure of the key K gives rise to a current in the complete circuit KBC, the magnitude of which is determined by the source of **EMF**, which is of the value of the pre-existing difference of potential between points BC, and by the total resistance in this circuit BCK.

It should be possible to determine the resistance of any portion of this circuit when the current is traversing it from a discovery of the difference of potential existing then between the terminal points of the resistance.

In this way it is possible to determine the longitudinal resistance between B and C, using as 'leads' to the terminals of this resistance the pieces of nerve AB, CD.

$$r = \frac{e}{E} R.$$

Where $E = .00448$, the pre-existing potential differences between points BC.

$R = 10,700 + 6,000 = 16,700$ ohms.

e = the newly-found potential difference between B and C.

= pre-existing potential difference between points AD—new potential difference between AD.

= $.01450 - .01305 = .00145$ Daniell.

$$r = \frac{e}{E} R.$$

$$= \frac{.00145}{.00448} \times 16,700 \text{ ohms.}$$

$$= 5,405 \text{ ohms.}$$

This value, obtained from this experiment, for the 'longitudinal resistance' between points B and C closely agrees, as a reference to the data of case (c) shows, with the value as otherwise calculated and used as the basis of the calculations made in case (a) and case (b), namely,

5,200 ohms.

The agreement of experimental results and prediction are so close in these experiments that one is inclined to place confidence in the measurements upon which the simple calculations depend, and one is, therefore, inclined to draw further conclusions from them.

In the first place, it is apparently obvious that the resistance of the longer stretches of nerve examined, as obtained from the WHEATSTONE bridge method, was also the actual resistance encountered by the nerve's own current when led through the outer path to the cross section, and so back through the nerve. Also, that a variation in the nerve's own current produced by the insertion of resistance in the outer path did not produce any effect upon this resistance. A reference to experiment A will show that the value for this resistance used in the calculations was the same, and led to the same satisfactory result, when the resistance in the whole circuit was varied from 135,000 ohms to 335,000 ohms; and when, therefore, the new current was in one case three times as great almost as in the other.

This longitudinal resistance apparently remains constant when the current traversing it is greatly varied, which would not be the case if any very large fraction of the resistance was due to polarization, and if, as is supposed, the polarization increased largely with the current.

This is a fact of considerable interest since, in dealing with the general problem of the electromotive phenomena of the nerve, it is necessary to know the nature and quantity of the electrolytes present in the nerve. The first guide to such knowledge is provided by measurements of electrical conductivity, and if these measurements are once acknowledged to be misleading by reason of special conditions present, then the main source of information is rendered valueless.

Measurements of electrical conductivity of nerve can be undertaken, by determining the resistance of a cut piece of nerve from cross section to cross section, in which it would seem that the error introduced by polarization would be absent, if it be assumed that all the polarization phenomena are due to the fact that stream lines pass from one constituent structure of the nerve fibre to another. For in this method of examination, when all the parallel constituent structures are traversed by stream lines parallel to them, this cannot occur, except as a negligible error due to artificially produced bends in the course of nerve fibres.

It is possible that even to such measurements exceptions may be taken, since there is some reason to fear the presence of a true 'longitudinal polarization,' the outcome of circumstances of physical structure not understood.

It is noteworthy, therefore, that in the measurements given above there is no reason to consider such a complication: so that the errors due to polarization are so small as to be negligible, and do not interfere with a practical use of these measurements as the basis of calculation.

The confidence obtained from such results has led to the institution of the measurements of conductivity in the next section, and their use as guides to the quantity of electrolytes contained in solution in the nerve, and, therefore, as assisting to form an opinion of the quantity of matter upon which all the electromotive phenomena of nerve depend.

MEASUREMENTS OF ELECTRICAL CONDUCTIVITY

The average resistance of the sciatic nerve of the cat is four thousand ohms per centimetre when given as based upon measurements of long stretches of nerve (five centimetres). The value of this statement (and it is of the orthodox character) is limited, in so far as it offers no basis for a real comparison of the resistance of the sciatic nerve with that of any other nerve of different average calibre, and also since it gives no information as to the varying nature of the resistances of the differing elements of structure entering into the composition of the nerve.

The first limit is only to be surmounted by a determination of the calibre, and this is a matter of some difficulty when use is made of nerves from which subsequently data of a different kind are to be sought, and which must not, therefore, be damaged in the process.

In the present series of measurements, this difficulty has been avoided by the use of an indirect method, which does not injure the nerve, and which affords results that are certainly not more than 5 per cent. in error.

The second limit it is not possible, for the present, to surmount, and the value of the information attainable is thereby greatly reduced. The reduction in value is not, however, so great as to leave the taking of these measurements merely an 'academic' interest; since the information obtained, scanty though it be, is from one point of view of extreme value. The nerve, being a 'moist conductor,' owes its conductivity to the solutions of electrolytes which it contains, and measurements of conductivity, therefore, can be used as guides to a knowledge of the quantity of electrolytes present in solution in the nerve, although giving no guide as to their relative distribution in its component parts. The information so obtained is by no means perfect, but is of value, since the limits to its accuracy are not such as to render the errors introduced more than a fractional part, even if a large fraction, of the true value which they tend to conceal.

The measurements, indeed, form the only means by which any approximate notion can be acquired of the total amount of electrolytes in solution, that is of the amount of matter which can partake in the production of the electromotive phenomena of the nerve; and it is obvious that in a study of these electromotive phenomena it is desirable to decide, even if roughly, the proportion which this amount bears to that of the total matter in the nerve.

MEASUREMENTS

The following data are taken from eleven separate experiments upon sciatic nerves obtained from eleven cats, from the bodies of which they were removed immediately after death. The measurements of resistance were made by an ordinary

'bridge' method, the circuit being so arranged that a current of less than $\cdot 01$ milli-ampère traversed the nerve at the time of measurement. The sensitive galvanometer used in the previous sections was also used in these measurements, and the determination was always finally made with this, and without a 'shunt.'

To avoid any error due to the presence of an injury current, the measurement was always repeated with the nerve arranged in a reverse direction, so as to cause this current to add to and to subtract from the measuring current; the values given are always the mean of two such measurements.

In each case given the piece of nerve, cut so as to be as near as possible five centimetres in length, was provided with a clean cross section at each end. It was arranged rectilinearly between two electrodes and upon a dry ebonite scale, and its length was accurately measured when thus in position. Immediately after the measurement of resistance the weight was accurately ascertained.

DATA FROM ELEVEN EXPERIMENTS

Experiments	Resistance in ohms	Length in centimetres	Weight in grammes
Experiment I	17,000	5·0	·287
„ II	14,200	4·8	·237
„ III	17,300	5·0	·239
„ IV	23,800	4·9	·201
„ V	15,900	4·8	·255
„ VI	17,800	4·8	·216
„ VII	23,500	5·0	·195
„ VIII	18,000	4·0	·145
„ IX	20,300	5·0	·236
„ X	20,700	4·5	·210
„ XI	26,000	4·9	·207
Average of 11 Experiments	19,500	4·8	·221

If, in these experiments, the determination of weight is treated as if it were a determination of volume, and the error so introduced is certainly less than five per cent., we have all the data necessary to determine the 'specific' conductivity of the nerve. The error would of course be eliminated by a correction for the specific gravity of the nerve, but this is difficult to obtain, owing to the peculiar behaviour of the nerve when immersed in solutions. Failing this determination of the specific

gravity the error is left uncorrected ; since it appears only once in the value of the specific conductivity, and is, therefore, not increased by multiplication to a higher power.

The 'specific' resistance of the nerve is taken as that of a closely packed pile of similar nerves, one centimetre in length, and offering a united cross section of one square centimetre.

In any single case such a 'specific' value may be found by dividing the value of the resistance by the length and multiplying by the ascertained value of the cross section.

$$\begin{aligned} \text{Specific resistance} \quad \dots \quad \dots &= r \times \frac{l}{\text{length}} \times \frac{\text{volume}}{\text{length}} \\ \text{or approximately} \quad \dots \quad \dots &= r \times \frac{l}{\text{length}} \times \frac{\text{weight}}{\text{length}} \end{aligned}$$

Thus taking the figures obtained as the average of the data of the eleven experiments—

$$\begin{aligned} \text{The specific resistance of the sciatic nerve of the cat} &= 19,500 \times \frac{.221}{4.8 \times 4.8} \text{ ohms.} \\ &= 180 \text{ ohms (approx).} \end{aligned}$$

Similar values given from the eleven separate experiments are—

I	195 ohms	VII	183 ohms
II	146 „	VIII	163 „
III	165 „	IX	191 „
IV	195 „	X	205 „
V	176 „	XI	165 „
VI	160 „		

Taking the average value of 180 ohms, it is of interest for purposes of comparison to compare it with the specific resistance of mercury at 18° C, with which value the resistance of solutions of electrolytes is commonly compared.

The specific resistance of mercury at 0° C.	94.07 × 10 ⁻⁶ ohms
The temperature co-efficient00077
∴ The specific resistance of mercury at 18° C.	95.4 × 10 ⁻⁶ „
The specific resistance of nerve	180
The specific resistance of mercury	95.4 × 10 ⁻⁶ „
	= 1.885 × 10 ⁶ „

Taking the 'specific conductivity' of nerve to be the reciprocal of its 'specific resistance,' as defined above, it is equal to—

$$\begin{aligned} &\frac{1}{1.885} \times 10^{-6} \\ &= 53 \times 10^{-8} \text{ in terms of mercury at 18° C.} \end{aligned}$$

The conductivity of nerve is now expressed in a form in which it can conveniently be compared to that of solutions of electrolytes. Thus, taking solutions of NaCl as our standards of comparison, we have the following determined specific conductivity of such solutions.¹

SPECIFIC CONDUCTIVITY OF SOLUTIONS OF NaCl

CONCENTRATION OF SOLUTION		SPECIFIC CONDUCTIVITY IN TERMS OF MERCURY
Gram molecules per litre	Grammes per cent.	
1.00	5.620	695.0×10^{-8}
0.50	2.865	378.5×10^{-8}
0.10	0.583	86.5×10^{-8}
0.05	0.292	44.8×10^{-8}

The specific conductivity of nerve is, therefore, approximately the same as that of a solution of sodium chloride of .35 grammes per cent. concentration. *In other words, the conductivity of the nerve would, so far as we are at present considering it, be adequately imitated by that of a saline solution occupying the same space, which was only half the strength of the ordinary 'normal saline' solution.*

It is of interest to compare the value so obtained with that of previous investigators. The data given by I. TEREG¹ are presented in a form most suitable for comparison. This author, in a general examination of the conductivity of the tissues and the modifications produced by changes of temperature, obtained a value for the resistance of nerves as follows:—The nerves were laid side by side in an accurately calibrated hard glass tube (diameter 12 mm.) The length of the tube used was 34.5 mm. The nerves were presumably packed closely in this tube and accurately cut to the required length. Amalgamated zinc electrodes were brought to each end of the tube and the resistance of the enclosed nerves determined.

The following values are given for this resistance:—

At 21° C.	700 ohms.
25° C.	670 „
32° C.	600 „
35° C.	570 „
39° C.	530 „
45° C.	460 „

1. Kohlrausch, *Wiedeman's Annal*, XXVI., p. 195.

1. *Archiv. fur Anat. : und Physiologie*, 1899, p. 318.

From these figures of TEREG's, and preferably from the value obtained of the resistance at 21° C, a value can be calculated for the so-called 'specific resistance.'

$$\begin{aligned}
 \text{Specific resistance} \quad \dots \quad &= R \times \text{cross section} \times \frac{1}{\text{length}} \\
 &= 700 \times \frac{\pi r^2}{l} \\
 &= 700 \times \frac{(\cdot 6)^2 \times 3 \cdot 14}{3 \cdot 45} \\
 &= 700 \times \frac{1 \cdot 13}{3 \cdot 45} \\
 &= 230 \text{ ohms approx.}
 \end{aligned}$$

The specific conductivity in terms of mercury therefore is (for data see previous example)—

$$\begin{aligned}
 &= \frac{95 \cdot 4 \times 10^{-6}}{230} \\
 &= 41 \times 10^{-8}
 \end{aligned}$$

A value which an examination of the table of specific conductivities of solutions of sodium chloride will shew is approximately the same as such a solution of the strength of .3 grammes per cent. The figures given by TEREG, therefore, closely agree with those found from the previous experiments, and like them set the electrical conductivity down at a very low value.

The modifications with temperature, as observed by him, are also of considerable interest, since they show this agency affecting the values in a quantitative manner, exactly agreeing with that known to occur with change of temperature in solutions of electrolytes.

Thus the conductivity at 21° C. is $\frac{1}{700}$, at 45° C. it is $\frac{1}{460}$. The alteration thus consequent upon a rise of temperature through twenty-four degrees is $\frac{1}{460} - \frac{1}{700}$ or $\frac{6}{8050}$. Treating this latter figure as a fraction of the original conductivity of $\frac{1}{700}$ it is seen to represent a rise in the original value of almost exactly fifty per cent., or *two per cent. per degree of temperature.*

The value of such a result is considerable, as is seen from a reference to the following quotation: 'The molecular conductivity of a given electrolyte depends in the first instance on the temperature, increasing almost without exception with rise of temperature, and mostly by about 2 per cent. per degree.'

The nerve, owing, as has been previously stated, all its electromotive phenomena, inclusive of electrical conductivity, to the solutions of electrolytes contained in it, is seen from the point of view of this modification with temperature to behave like any solution of electrolytes. This is a fact worthy of consideration when, as has sometimes been attempted, assumptions are made as to the mobility of the particles contained in it with the conduction of an electrical current other than that of the motion of ions in solution.

If the electrical conductivity of nerve seems disappointingly small when it is sought to discover as its main function that of an electrical conductor, and when the prominence of the electrical phenomena discovered in it is considered, it is no longer so when a glance is taken at the known chemical constitution of the nerve. Thus, taking the figures collected in HALLIBURTON'S¹ article on the chemical constitution of nerve, sciatic nerves contain 61.3 per cent. of water and 38.7 per cent. of solids.

The solids are given in the following estimation made from human sciatic nerve :—²

SOLIDS OF HUMAN SCIATIC NERVE

Proteids	36.80 per cent of the total solids.
Lecithin	32.57
Cholesterin and fat	12.22
Cerebrins	11.30
Neurokeratin	3.07
Other organic matters	4.00
			99.96

None of these bodies, which are arranged here, contributing to the solids of the nerve offer much prospect of a capability of acting as electrolytes: the only substances which would seem in this company to be characterized as such, the inorganic salts, are omitted from the table presumably from a failure to estimate the unimportant constituents. To form an estimate of the amount of inorganic salts present, we are compelled to use the quantity estimated as present in the white matter of the brain, namely, .57 per cent. of the total solids, or in the spinal cord forming 1.1 per cent. of the total solids.

These figures, while providing no exact guide, might lead us to infer that the inorganic salts of nerve formed .3 or .4 per cent. of its mass, a value which is suggestively similar to that of the total electrolytes as roughly estimated by the conductivity method.

More point is given to these figures by the fact that the inorganic salts of nerve are not expressible as so much sodium chloride, a large quantity of potassium salt being also present, the electrical conductivity of which is greater. To explain the conductivity of nerve in terms of its inorganic salts, it is, therefore, necessary only to find present a quantity of these less than .3 per cent., and there seems every possibility of doing so.

The small value of the electrical conductivity of nerve has long been appreciated, although few attempts have been made to exactly determine it. The fact in its gross form was, subsequently to the discovery of the electrical current by GALVANI, made of

1. Schafer, *Textbook I*, p. 116.
2. Moleschott, *Physiol. Chem.*, p. 335.

great polemical use as a conclusive argument against the advisability of seeking a purely electrical function for nerves in the body. It has, however, also been repeatedly pointed out that the nerve is not a homogeneous conductor, and that measurements of gross conductivity give no information as to the conductivity of the very different longitudinal elements of structure which compose it. From such a point of view it is seen, especially if one of these structural elements is assumed to have a semi-insulating character (very low conductivity), that such gross measurements may be most misleading if used in any way to limit the possibilities of conductivity of any individual element of structure.

Given a tissue of extremely high electrical conductivity, arranged in exceedingly fine threads within the general mass of poor conductors, its presence might be totally unsuspected, and might even be said to be concealed by the general low conductivity found.

It is of interest, therefore, to consider the only case in which careful investigation has led its authors to the conclusion that nerves are after all characterized by a high electrical conductivity much greater than that of any other of the tissues.¹

These authors, ALT and SCHMIDT, have, by use of a new and peculiar method, compared the resistance of different animal tissues, and find them arranged in the following order; in which, it will be seen, the standard of comparison is an arbitrary one, the resistance of muscle—

Nerve	0.17
Heart muscle	0.86
Muscle	1.00
Blood	1.00
Aponeuroses	4.41

etc., etc.

Such a statement places nerve in the position of being as an electrical conductor six times superior to muscle in opposition to the common view, that it is, on the contrary, inferior to it.

The experiments bear every mark of careful and repeated work, and the conclusion to which the authors have come seems, therefore, to be fully justified by them. The method is, on the other hand, quite new, and can only be adequately criticized by a physicist. In this method the tissues placed within and filling a glass tube of standard size were placed in line with other conductors to form an alternative path for the conductor of 'FRANKLIN currents from a HOLTZ influence machine.' The other path was a spark gap.

The method would seem to depend upon a comparison of the resistance of the tissue and the resistance of a column of air, which would seem to be an absurdity. As a matter of fact, probably this is far from being the case, the spark conducted

1. Alt & Schmidt, *Halle Effügers Archiv*. LIII, p. 575, etc.

through the air being conveyed not by particles of the air but by charged particles discharged from the conductor, and the real resistance of the air gap is not, therefore, open to calculation.

The authors believe that a high general conductivity is revealed in the nerve by this method, since, in their opinion, the method eliminates all error due to polarization. It must be admitted that such a revelation would place the longitudinal polarization of nerve in a position of extreme importance as an agent capable, under ordinary circumstances, of masking 90 per cent. of the electrical conductivity of the nerve. This extreme value of the longitudinal polarization has no direct evidence in support of it, and all the available evidence (as that obtained in the last section) points to the opposite conclusion, that polarization, though characteristically present and of importance, is not able to mask more than a fraction (less, say, than one-fifth) of the gross conductivity, and adds but a small fractional addition to the measurable resistance.

The difference found between nerve and the other tissues must have some reason, even if this is not expressible in the terms chosen by the authors, and there seems the remote possibility that the method has revealed the possession by the nerve of extremely fine paths of high specific conductivity.

THE PHYSICAL STRUCTURE OF THE NERVE

The pre-existing structures of the nerve are such as to primarily determine the fact that local injury is productive of an injury current: and this is true whether the differential distribution of electrolytes giving rise to it is (1) also pre-existent, or (2) the result of chemical change, a secondary consequence of injury.

To explain fully the intended meaning of this statement it is necessary to briefly consider the nature and arrangement of materials in the nerve from a purely physical point of view. It is not, and never has been considered, sufficient to dwell solely upon details of histological structure, and to read into them an appropriate physical meaning, and it is at the present date more obvious than ever that this is not the primary method of examination. The data of primary importance are provided by a knowledge of the manner in which the structures of the nerve behave, when the electrolytes contained in solution in them are set in movement by a source of electromotive force; or it might also be said by the manner in which the electrolytes, when diffusing, give rise to a source of electromotive force.

From such a point of view an intimate acquaintance is necessary with:—

- (a) The conductivity of the nerve.
- (b) Secondary features of its conductivity, such as the polarization phenomena.
- (c) The injury current.

The consideration of the last aid to knowledge, the injury current, is, although held to be the most important, abandoned in this section as begging the question set in the research.

The facts which have been determined by purely physical methods of examination are contained in the statement that the nerve behaves as a core model, and that a core model is always a complex conductor composed of materials of at least two different specific conductivities, arranged cylindrically, the one surrounding the other.

Practically it has always been found necessary to place in the core of the core model a material of higher specific conductivity than that of which the mantle is composed, and this fact may be used as an argument that the core of the nerve fibre is of higher specific conductivity than its sheath (it being universally acknowledged that the comparison between core model and nerve trunk can, without fallacy, be used as if in reality a comparison between the core model and nerve fibre).

The argument is not as good a one as might at first sight appear, since this property of the core model structure is necessitated by a characteristic which may be peculiar to it, and may not adequately represent a condition present in the nerve. For the successful imitation of the physical characteristics of the nerve by the core model is due to the acknowledged fact that, although commonly only composed of two materials, it opposes resistances of three kinds to the passage of an electrical current through it:

- (1) the surface resistance of the mantle ;
- (2) a high resistance at the surface of separation of core from mantle, due to internal polarization, and therefore only existing during the passage of the current ;
- (3) the internal resistance of the core.

Three conditions are, therefore, obtained in the core model by the presence of two materials: but in order to obtain these three conditions from two materials, it is necessary that an extraordinary difference should be found between them. The conditions are only adequately so obtained by using a metallic conductor as the core, a dilute moist conductor as the mantle. The high specific conductivity of the core substance of the model is therefore possibly only an accidental attribute, due to the fact that a metallic conductor is necessarily chosen to obtain the polarization resistance.

The nerve fibre is, however, throughout a moist conductor. It seems an absurd, but is a necessary, statement that no metallic conductor exists within its core. The nerve, therefore, may indeed imitate the three conditions present within the core model carrying a current, it cannot, however, imitate them so successfully as to be composed of two analogous sets of materials. It is necessary to consider how the three conditions can be obtained by the use of moist conducting material alone without the assistance of the metallic core. The result of such a consideration is of extreme importance, for it has been found impossible to represent the three conditions by the use of moist conducting material without making use of three materials of different specific conductivity,

- | | | | | |
|-------------------------------------|---|---|---|------------------------|
| (1) of fair conductivity | . | . | . | mantle. |
| (2) of bad conductivity | . | . | . | intervening structure. |
| (3) of conductivity better than (1) | . | . | . | core. |

To carry the lesson learned from the core model to the structure of the nerve fibre we must, therefore, seek in the nerve fibre and the solution covering its surface for the analogues of these three different materials of different specific conductivity.

It is to be noted that in such a core model polarization resistance developed during the presence of the current (and only present then as in the case of the two material core model) is now of secondary importance. A pre-existing resistance now partially at least occupies its place, and the polarization which occurs is only a secondary addition to this pre-existing resistance.

The statement made above, though so briefly given, has involved years of controversy and experiment upon the part of several investigators, notably HERMANN and GRÜNHAGEN. This controversy has been now brought to a clear termination by the introduction into the subject of knowledge of the possibilities, within which the conduction of electrical currents by moist conductors is limited, by the 'electro-chemist' NERNST.

The statement of electro-chemistry is definite. Polarization cannot occur between two moist conductors (solutions of electrolytes) unless they are separated by a physical membrane (pre-existing high resistance). The introduction of the third material is, therefore, a necessity, and its nature is even to a certain extent defined, for the term 'physical membrane' implies a material which habitually acts as a barrier, limiting material particles moving in diffusion processes, and also, as in this case, the particular particles 'Ions' which are set in motion during the passage of an electrical current.

Practical experience is, therefore, amply confirmed by theoretical consideration.

Definite as is the statement of 'electro-chemistry' as well represented by NERNST, it is of interest that a similar statement was also simultaneously made by a physiologist making use in other fields of electro-chemical data and methods. This statement is, unfortunately, hidden away from the special literature of 'muscle and nerve,' inasmuch as it appears in an article on blood and blood corpuscles. It seems therefore pardonable to quote it in some detail. G. N. STEWART discovered the important fact (amply confirmed by its simultaneous discovery by ROTH, BUGARSKY, and TANGL, etc.) that the limiting surface of the red corpuscle offers an extremely high electrical resistance, when immersed in its natural surrounding fluid, the blood plasma.

The ions contained within the blood corpuscles in solution are capable of free movement within the confines of their walls, as is shewn by the osmotic pressure which they are known to be capable of exerting upon them. Conduction of an electrical current would therefore also freely take place within the corpuscular walls. Observations therefore which place the corpuscles in the position of poor or non-conductors can be used in evidence against the conductivity of the walls themselves, since they cannot be directed against the contained solutions.

Ions moving through the blood plasma in the orderly conduction of an electric current are stopped by the surfaces, and do not penetrate the mass of the red corpuscles. In other words, the limiting surfaces are only partially permeable membranes, even if not strictly semi-permeable membranes; and this statement so made is amply confirmed by evidence of a different kind. For such experimental evidence is only an additional confirmation of a long well-known fact, that the inorganic salts of the plasma and of the blood corpuscles are not the same, and of the corollary which this fact implies, namely, that the walls of the blood corpuscles form barriers to diffusion processes between the solutions within and without them.

The fact is, however, capable of extension to other tissue elements such as nerve fibres, which are only finely-drawn processes of cells also containing inorganic salts within them differing, in proportional amount at least, from those contained in the solutions without. The extension of the fact enables the enunciation of an apparently unobjectionable general statement, that all cell walls (walls of nerve fibres included) are possessed of this property of limited permeability to the particles in motion in the solutions surrounding them, and this no matter what be the force under the action of which these particles are moving. The general statement therefore includes the movement of ions carrying an electrical current.

From such or similar consideration G. N. STEWART¹ comes to the following conclusion as to the value of the polarization resistance stated by HERMANN to be found on the surface of nerve fibres during the passage of an electrical current:—

‘But if nerve fibres are surrounded by an envelope whose specific resistance is much greater than that of the contents of the fibre, there must be a very abrupt change of potential as we pass along current lines that cut the envelope, and the surface of the envelope may therefore become strongly polarized. The fact discovered long ago by HERMANN, that the apparent conductivity of nerve across the fibres is many times less than its conductivity in the longitudinal direction, although explained by him as due to the relatively great capacity for polarization of the nerve when the polarizing current passes transversely across it, receives a more natural explanation if we suppose that the nerve fibres are surrounded by badly-conducting envelopes. Of course if this is the case, a part of the apparent excess of transverse resistance may still be due to polarization, but not the whole of it, nor probably any large proportion of it.’

Such a view is a repetition of GRÜNHAGEN’s original position, that the major portion of the transverse resistance was due to a pre-existing high resistance envelope, separating the core of the nerve fibre from the outer ‘nutritive fluid.’ It is based like it upon a knowledge of the histological structure of the nerve, but whereas GRÜNHAGEN’s envelope was peculiar to the nerve as its fatty sheath, and was, therefore, rejected on appeal to the similar existence of a property by muscle where such a sheath is non-existent; STEWART’s envelope is the common property of the nerve fibre and of all cells. The envelope may include the neurilemma and the myelin sheath or both. Its thickness is not of such importance as its quality, and its quality is the common property of all limits to cellular structures no matter how microscopically minute they may be.

Such opinions based upon the one hand upon an intimate acquaintance with the properties, and the limits to the properties, of moist conductors (NERNST), and on the other hand, upon an intimate acquaintance with the physical value of histological structures, come to the same conclusion. The nerve fibre, covered with its surrounding solution, can only be successfully imitated by a concentric arrangement of three materials of different specific conductivity, and the theoretical considerations indicate amply that no simpler arrangement is possible.

1. G. N. Stewart, *Journal of Physiology*. XXIV, p. 212-3

The physical structure of the nerve fibre is therefore a 'core model' structure, and necessarily comprises—

- (1) an outer solution of electrolytes.
- (2) a partially permeable membrane.
- (3) an inner solution of electrolytes.

Having come to a definite conclusion, that the three conditions of conductivity inferred as existing concentrically in the nerve fibre must be the outcome of a concentric arrangement of at least three different structures, we are in a position to ask whether the statement is still justified that the most internal structure has a specific conductivity of great comparative importance.

Is conduction of an electric current by a nerve trunk a phenomenon mainly occurring in the axis cylinders of the nerve fibres? If so, is this to be explained by the presence of a relatively greater volume of an uninterrupted (by membranes, etc.) solution of electrolytes here than elsewhere in the nerve, or is it to be explained by the presence of a solution of small volume but great concentration and conductivity?

The question seemed answered in the affirmative by a reference to the metallic core of the metallic core model. Such a core model does not, as has been stated, however, adequately represent the distribution of structures in the nerve fibres. Core models, however, such as GRÜNHAGEN'S, have been frequently used, which attempted more completely to imitate the distribution of structures in the nerve, being entirely composed of moist conductors. It is of great practical interest, that in such models it has always been found necessary to make the internal solution of relatively great specific conductivity before a resemblance was experimentally found between the nature of the electrical conductivity of the model and the known nature of the conductivity of the nerve, which it was designed to imitate.

The characteristic feature of electrical conductivity in nerve is provided by the electrotonic currents *and by their distribution*. The electrotonic currents can be imitated by the core model, the distribution of such currents in the nerve can only be imitated by a core model in which the internal solution is of high specific conductivity.

This experimental fact is at least an indication which cannot be neglected, and is universally recognized as such. It is taken to mean that the axis cylinder of the nerve fibre is a better conductor than the tissues which ensheath the fibre, and, therefore, that more electricity is conveyed along the axis cylinders than is simultaneously carried by the other tissues of the nerve, when both are carrying an electrical current.

It has been taken, however, by HERMANN to mean that more conducting material is present in the axis cylinder and not that the conducting material present is of a better kind.

Such a conclusion is, however, difficult to follow ; since in the first place there is small ground for entertaining a belief in a relatively greater volume of the solutions of electrolytes placed there, and in the second place such a conclusion is in contradiction to the fact that in the model the conditions were obtained otherwise, namely, by the use of an internal solution of greater specific conductivity and not of greater volume.

One is, therefore, justified in stating, that the only obtainable evidence is in favour of the view that the solutions of electrolytes present within the axis cylinder are of greater specific conductivity than the solutions present elsewhere in the nerve trunk.

Since such a greater conductivity can only be explained in one of two ways, namely, that the electrolytes in the internal solution are different in nature or greater in concentration than those found in the external solution ; such an inference may be used to point to one of these two conditions as of probable occurrence. and this is of great importance from the point of view of the 'injury current.' Immediately it is granted that the internal and external solutions are not the same, it becomes almost necessary to assume that the rupture of such a compound conductor would give rise to new processes of diffusion, and so to an 'injury current.'

Even if we abandon this most probable view, that there is a pre-existing difference between the solutions, and for the time being suppose that the internal and the external solution are one and the same in nature and in concentration ; still we cannot afford to neglect the importance of this tubular membrane, capable of maintaining a difference between the solutions, should any new cause for such a difference arise.

Let any chemical change occur in the matter within the tubular diffusion obstacle, and lead to the formation of new electrolytes and to their appearance in the solutions therein contained ; at once is seen the possibility that they may be confined to this situation by the enclosing membrane.

Let, for example, carbonic acid be produced from the destruction of some complex organic body in the axis cylinder, then it is conceivable that this substance might diffuse with greater ease along the track of the internal solution than through the membrane into the external solution. At once a difference is created between the two solutions, and, were the nerve ruptured, then at the injury the carbonic acid would have its first chance of freely escaping from the internal into the external solution.

Granted the core model structure of the nerve fibre and the existence of this tubular surface of separation of the solutions contained in the nerve, we are at once presented with an important factor determining the origination of an 'injury current.'

The first effect of injury is to disturb this barrier between the internal and external solutions, and whenever differences already exist between them, to give rise *ipso facto* to a 'current of injury.'

A second effect of injury may be to lead to new chemical change, and to new differences between the two solutions. Even if so, it is extremely probable that the localization of the resulting electrical phenomena to the region of the injury may be a consequence, not of the localization of the chemical change, but of the injury to the tubular membrane.

In considering the physical structures of the nerve, therefore, the greatest stress is laid upon this separation of structures into 'internal' and 'external,' and upon the presence of the limiting surface which determines this separation.

The experiments recorded in the subsequent sections of this paper have been devised to test the opinion that this is a matter of primary importance.

REPLACEMENT OF THE EXTERNAL SOLUTION OF THE NERVE BY WATER

EXPERIMENTS ON NORMAL, ABNORMAL, AND DEGENERATED NERVE

In the last section it was stated that the main characteristic of nerve of interest from the point of view of the injury current was the presence of 'membranes,' which confine the important structures of the nerve cell processes from too familiar contact with the surrounding lymph. Such an arrangement is by no means peculiar to nerve, but seems to be the common property, in varying degree, of every cellular structure. The peculiar characteristic of nerve is the longitudinally unbroken continuity of its constituent parts, and their arrangement side by side in the nerve trunk in parallel tubular compartments. In a sense this peculiarity is shared with muscle; but in that case there is a secondary transverse segmentation which is not obvious in nerve, and also the limits of the peculiarity are there narrowed by the incomparably shorter length of the muscle fibres.

If it is believed that the internal solution of electrolytes found within cells in general is not the same as that which is found bathing their external surface, then the case of the nerve cell process of the nerve trunk offers itself as the most suitable for the testing of this opinion. For such a difference must give rise upon rupture of the membranes to diffusion processes, and consequently to differences of potential; and the prolonged surface of the nerve cell process obviously offers the best case for the examination of these electrical differences.

If such electrical differences as are found in the phenomenon of the injury current are to be totally explained in this way, they should be capable of modification in a manner entirely the same as that which would be expected from a process of diffusion. The value of a diffusion process depends upon the ratio between the concentration of the two solutions in contact, between which diffusion is taking place, and it can be greatly increased by diminishing the concentration of the weaker solution. Let us, therefore, reduce the concentration of the external solution of the nerve trunk to a minimum, the value of the diffusion process consequent upon rupture of the 'membranes' (which confine the internal solution) should be greatly enhanced, and with this there should also occur a great increase in the injury current.

Such a modification of the conditions of the nerve trunk is easily obtained by immersing it in water. For there is no reason to doubt that the primary effect of such an immersion is the extreme dilution of the external solution; although, as a later consequence, a dilution of the internal solution must also inevitably occur. This differential modification of external and internal solution is the usual result of immersion in solutions, and is a fact continually taken advantage of in the impregnation

of tissue with dyes for purposes of histological research. In such researches the artifice of immersion is freely used to remove some impregnating solution from the external surface, and to leave it within the internal solutions of the cellular elements of the tissue. It is an occurrence, however, which does not require for its confirmation such evidence as this, if it be granted* that the limiting surfaces of cell, as shewn by the osmotic pressure exerted by the solutions in them during immersion in water, are barriers which limit the extent to which diffusion can take place through them. For it at once follows that it is easier to remove particles in solution outside the barrier, than those which are protected by such obstacles to their removal. Moreover, this differential modification by immersion in water is seen to be the converse of a modification of which no one will deny the actuality, namely, the easier access of an impregnating solution to the external solutions of the nerve than to the axis cylinders of its nerve fibres.

Granted that an immersion in water affects first the external solution, the modification in the injury current produced by such an artifice is of great interest. *Immersion in water always very sensibly increases the injury current of nerve.* The result of the experiment is entirely in agreement with the anticipation, which foresaw such a result following upon the dilution of the external solution; and in so far as it is a confirmation of this, it may itself be used to strengthen the evidence in favour of the great importance of the core model structure of the nerve.

Nor is this the limit to the interest of the information which can be obtained from such experiments. There is no circumstance under which nerve shews an injury current, that the current is not increased by an immersion in water. But further, whereas nerve can be placed in, what is ordinarily considered, such a debilitated condition that it shews no injury current, it sometimes happens that even then an immersion in water will evoke from it an injury current as great as that which can be obtained by the same artifice from the most 'vigorous' nerve. These latter conditions are, in fact, such as to justifiably provoke the following statement:—*Even when all the solutions in the nerve, external and internal, have been brought by processes of diffusion to a common level, then an immersion in water is of itself productive of such a new difference in concentration of the external and internal solution as to reproduce, and to reproduce to its full extent, the phenomenon of the injury current.* This statement is based, as will be seen, upon the examination of the injury current of degenerated nerve. Such a nerve removed from the body and provided with a new cross section may shew no injury current, and yet an immersion in water may reveal a current as great as that obtainable from a healthy nerve taken from the same animal and subjected to the same artifice.

In such a nerve there is reason to believe that the tubular limiting 'membranes' are yet intact, their contents, on the other hand, are gravely altered. There is still, in such a nerve cell, substance limited by the neurilemma: although the cell substance

* See Preliminary communication, *Proceedings Royal Society*. J. S. Macdonald, vol. lxvii, 315-320.

is not that of the nerve cell process, the myelin and the axis cylinder being broken up and discontinuous. The usual explanation of the presence of a small injury current in such nerves is a 'vital' one, and in terms of the discontinuous fragments of the axis cylinder. On such lines, if it is possible to explain a small current, it is quite impossible to explain a phenomenon as great as that obtainable from the intact nerve.

The explanation which is offered now in the terms of the statement given above, is based upon the fact that the value of a diffusion process (or of a potential difference caused by one) is dependent upon the ratio between the two solutions, the same value being obtainable by the contact of several sets of solutions occupying different places in the range of possible concentrations.

If it were possible to obtain pure water free from dissolved matter, the greatest possible difference between two solutions of approximately similar concentration would be between such pure water and a water in which there was only a slight trace of added electrolyte. The difference given by such a combination would be greater than that obtained by 'contrasting' impure water with any obtainable solution, for in the first case the ratio is infinitely great, its denominator being zero. Such a combination could, however, only exist for an infinitely short space of time, since contact with the impure water would rapidly soil its theoretically pure neighbour. Similarly, when there is any great difference such as this between the two solutions in contact, the difference is rapidly diminished by what we may term the soiling of the standard of comparison—the more dilute solution. Considerations such as this render a satisfactory explanation feasible for the practical impossibility of obtaining infinitely great differences of potential between solutions in contact.

In the extreme case when water is offered as the standard of comparison to any solution, the actual result obtained is always smaller by reason of this error, and the greater the concentration of the contrasted solution the greater the diminution due to the error.

Granted in the experiments quoted that the tubular membranes are intact or still serviceable. Granted, also, the small current found is to be explained as due to the existence of only a small difference between the internal and external solution. There is, then, every reason to conclude that it might be possible to obtain as great a current from such a nerve after an immersion in water as from a normal nerve. For in neither case, when the observation is taken, is the outer solution actually replaced and maintained as replaced by water; and the depreciation in the value of this standard of comparison (water) is likely to be greater when the internal solution is of considerable concentration.

It is worthy of note that, once granted the schematic structure which is claimed for the nerve, every difference between its internal and external solutions is liable to lead to an injury current, the direction and amount of which is determined by the

difference. The only circumstance which can lead to the absence of an injury current is the temporary equality (in essential particulars) of the two solutions. The circumstances which determine a final disappearance of the phenomenon are:—

1. The destruction of the physical characteristics of the tubular membranes.
2. And the total removal of all electrolytes from both solutions by a prolonged washing of the nerve in water.

One other point is also worth attention. Some reason has been given for concluding that the internal solution of electrolytes is the more concentrated of the two. Granted the correctness of the view which is here taken as to the causation of the injury current, then these experiments with water are in confirmation of such an opinion. *The fact that an increased dilution of the external solution leads to an increase of the phenomenon may be taken as confirmative of the opinion that the phenomenon is due to the comparative dilution of the external solution.*

The measurements of resistance given in the following experiments were undertaken as indices of the extent to which the immersion in water succeeded in washing electrolytes from the nerve.

EXPERIMENT (TAP WATER)

VAGUS NERVE OF DOG

Piece of right vagus ten centimetres long. Removed immediately after death. The potential differences given were measured between the upper cross section and points upon the longitudinal surface.

Points (1) (2) (3), etc., refer in each case to a point on the longitudinal surface distant 1, 2, 3, etc., centimetres from the upper end of the nerve. Point (10), therefore, is the second cross section, and the difference between the two cross sections recorded under this heading is here as in the other experiments marked by an asterisk.

Potential Differences			Average Values
Point (1)	·00248	·00296	27.2×10^{-4} D.
„ (2)	·00300	·00328	31.4
„ (3)	·00324	·00328	32.6
„ (4)	·00348	·00328	33.8
„ (5)	·00364	·00328	34.6
„ (6)	·00364	·00328	34.6
„ (7)	·00364	·00348	35.6
„ (8)	·00360	·00336	34.8
„ (9)	·00312	·00304	30.8
„ (10)*	·00008	·00008	*00.8

Points marked thus * are in each case the second cross section.

Below are given the resistances measured from the upper of the nerve to each point observed.

Point (1)	...	10,600 ohms	or	10,600 ohms per centimetre
„ (2)	...	17,900 „		8,950 „ „
„ (3)	...	26,000 „		8,660 „ „
„ (4)	...	33,100 „		8,520 „ „
„ (5)	...	41,500 „		8,300 „ „
„ (6)	...	50,700 „		8,450 „ „
„ (7)	...	59,600 „		8,510 „ „
„ (8)	...	69,100 „		8,640 „ „
„ (9)	...	78,700 „		8,700 „ „
„ (10)*	...	82,200 „		8,200 „ „

The nerve was now removed from the moist chamber and placed in a large quantity (2 litres) of *tap water*, in which it was immersed for ten minutes. At the end of this time the nerve was removed, dried in filter paper and replaced in position in the moist chamber. A re-examination was then made of the potential differences between the upper cross section and points upon the longitudinal surface.

Potential Differences			Average Values
Point (1)	·00673	·00568	$62.0 \times 10^{-4} D.$
„ (2)	·00766	·00673	71.9
„ (3)	·00935	·00800	86.7
„ (4)	·01030	·00937	98.3
„ (5)	·01214	·01096	115.5
„ (6)	·01307	·01214	126.0
„ (7)	·01650	·01500	157.5
„ (8)	·01404	·01325	136.4
„ (9)	·00713	·00739	72.6
„ (10)*	0	0	0

Points marked thus * are in each case the second cross section.

The potential differences between the same points and the other cross section were next determined.

Potential Differences			Average Values
Point (1)	·00568	·00436	50.2×10^{-4} D.
„ (2)	·00634	·00528	58.1
„ (3)	·00726	·00634	68.0
„ (4)	·00837	·00726	78.1
„ (5)	·00924	·00800	86.2
„ (6)	·00977	·00919	94.8
„ (7)	·01135	·01096	111.5
„ (8)	·01016	·00977	99.6
„ (9)	·00488	·00488	48.8

Finally, the resistances between each point and the upper cross section were again taken to determine the alteration produced in them by immersion in water.

From Upper Cross Section to	RESISTANCE	
	In ohms	In ohms per centimetre
Point (1)	13,500	13,500
„ (2)	30,000	15,000
„ (3)	39,300	13,100
„ (4)	51,700	12,950
„ (5)	61,700	12,340
„ (6)	92,400	15,400
„ (7)	99,700	14,240
„ (8)	109,000	13,620
„ (9)	120,000	13,300
„ (10)*	110,300	11,600

Points marked thus * are in each case the second cross section.

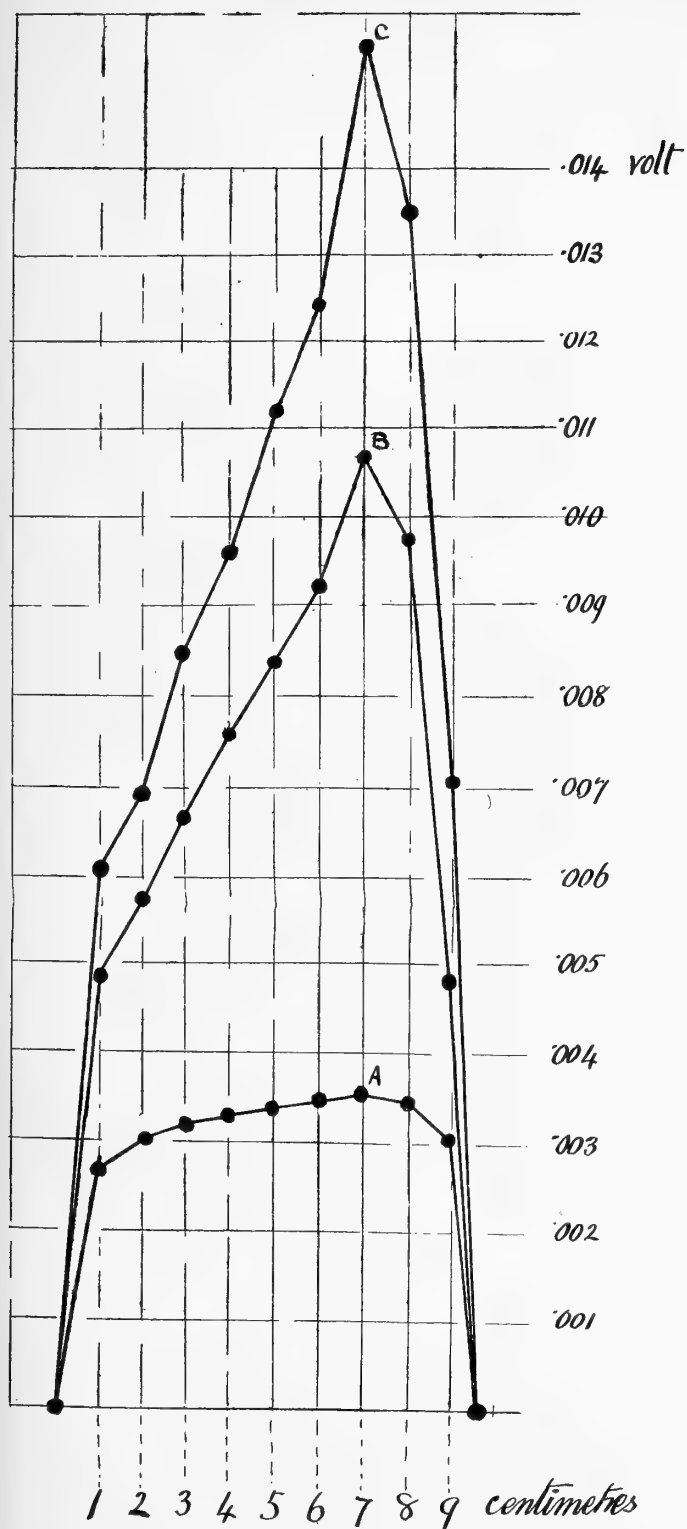


FIG. W

EXPERIMENT (TAP WATER)

(Fig. W)

A is the curve of distribution of potential upon the nerve when removed from the body. It will be seen that, in all essential details, it is similar to the curves given in the preceding section upon the 'current of injury.'

C is the curve of distribution of potential after the immersion of the nerve in tap water (p. 277). The point of reference was in this case, the upper cross section.

B is the similar curve obtained later (it is, therefore, lower). The point of reference in this case was the lower cross section (p. 278).

Attention is drawn to the position of the maximum point in each of the three curves A, B, C.

In this experiment the resistance of the nerves is increased from 82,200 ohms to 110,300 ohms by the immersion in water, an increase which can immediately be assigned to the removal of electrolytes from the nerve. There is also an indication in the data that the superficial resistance is more affected than the 'longitudinal resistance,' and, therefore, some confirmation of the otherwise amply justified belief that the electrolytes removed from the nerve come largely from the surface solutions.

Support for this last statement is found in the measured resistance of points (9) and (10). In the measurements of the resistance after immersion the paradoxical result is obtained that the resistance of 10 centimetres of nerve is 110,300 ohms, whereas the resistance of 9 centimetres of nerve is 120,000 ohms.

The resistance of the 10 centimetres is, on consideration, obviously less, because taken from cross section to cross section and not through the transverse resistance, whereas the 9 centimetre resistance includes some of this transverse resistance. In this case, also, the transverse resistance must be relatively much greater than before; since in the resistance measurement taken before immersion the influence of the same factor can be detected, but not present in sufficient force to produce the same paradoxical result.

Attending this loss of electrolytes, inferred to be mainly from the 'outer solution,' there is a great increase in the potential difference between longitudinal surface and cross section.

(Before, 35.6. After, 160.0)

an increase of more than four times the original value.

The curves of distribution of potential taken are of interest, in so far as they are typically asymmetrical. The curve taken after immersion in water is very obviously so, and the repetition of the curve taken from the second cross section, which was undertaken to prove that the asymmetry was not the artificial product of the order in which the observations were taken, is an ample confirmation of this.

If the curve taken before immersion is examined it will be seen that the asymmetry was already present before the immersion in tap water, that the maximum of the curve in fact remains in a position unaltered by the modification.

It is worthy also of incidental notice that at the time when the asymmetry was most marked, after the immersion, there was no potential difference between the two cross sections to account for it; that also the repetition of the same curve by a subsequent examination taken from the second cross section as reference point leaves no possibility of considering the asymmetrical form of the curve as the outcome of the mode of observation; that the measurements of resistance do not reveal any variation in the calibre or in the specific resistance of the nerve in different portions of its length which could make 3 centimetres of the nerve on one side of the maximum point equivalent to 7 centimetres upon the other; and finally that a consideration of the form of the curve leaves little room for the suggestion that its irregular form is due to the presence of accessory local injuries.

It may, from the experience of many similar experiments, be definitely said that the results of this experiment, whatever be the assumption to test which it was made, or whatever the explanation offered, conclusively demonstrates an experimental fact capable of constant repetition, namely, that a short immersion in water leads to a great increase in the value of the potential difference between longitudinal surface and cross section of the nerve, and even, since the resistance is not proportionately increased, to a great increase in the injury current.

Nor is this effect of an immersion in water confined to an effect upon nerve in any particular condition, for it is capable of being repeated upon nerves with very different previous histories. One condition, however, in which it is unattainable is worthy of especial attention, a long continued previous immersion in water, for in this case there can be no question of the meaning of the exception. *When the electrolytes in solution are all removed by processes of diffusion there can occur no further electrical phenomena.*

The following are briefly recorded further examples of this effect of an immersion in water :—

(1) A NERVE FRESHLY REMOVED

Sciatic nerve of cat on removal	·024 Daniell.
After 10 minutes in tap water	<u>·053</u> „

(2) A STALE NERVE WITH DIMINISHED CURRENT

Phrenic nerve of dog on removal	·0060 Daniell.
After 3 hours in the moist chamber	·0008 „
After 10 minutes in tap water	·0101 „

(3) A NERVE WHICH HAS BEEN PLACED IN STRONG SALT SOLUTIONS

Sciatic nerve of cat on removal	·0152 Daniell.
After 1 hour 40 minutes in 9% NaCl	·0010 „
After 45 minutes in tap water	·0224 „

(4) NERVES WHICH HAVE REMAINED LONG IN THE BODY OF A DEAD ANIMAL

(a) Sciatic nerve of dog removed 24 hours after death	·0001 Daniell.
After 10 minutes in tap water	·0040 „

(b) Dog dead 3 days and 2 hours, during two days of which the body was placed in a refrigerator, and then for 24 hours was allowed to lie in a warm room (August).

Sciatic nerve on removal	·001 Daniell.
After 5 minutes in tap water	·003 „

A short immersion was chosen under these circumstances, for it has been found that a long immersion in such a case results in a reversal of the injury current, a matter which will be further treated later.

(4) NERVES WHICH HAVE REMAINED LONG IN THE BODY OF A
DEAD ANIMAL—*continued*

(c) Cat dead 7 days. Placed in the refrigerator during the whole of this period, except the first 4 hours.

Sciatic nerve on removal	·003 Daniell
After 10 minutes in tap water	·008 „

(5) DEGENERATED NERVE

The two following experiments are recorded in some detail, since the results obtained in them are of an obvious interest. The main facts have been previously recorded* and are here quoted, the details being given in an appendix.

(a) VAGUS NERVE OF DOG

Preliminary operation. 1 centimetre of nerve excised at upper, and 1 centimetre at lower limit of nerve in the neck.

Examination nine days afterwards. The animal was killed and the degenerated nerve immediately removed

...	·000 Daniell
After 25 minutes in tap water	·020 „

(b) SCIATIC NERVE OF DOG

Preliminary operation. 1 centimetre of nerve excised.

Examination twelve days afterwards. The animal was killed and the degenerated nerve immediately removed

...	·003 Daniell
After 40 minutes in tap water	·023 „

* See Preliminary communication, *Proceedings Royal Society, loc. cit.*

APPENDIX

EXPERIMENTS UPON DEGENERATED NERVE

EXPERIMENT I

VAGUS NERVE OF DOG

Professor SHERRINGTON most kindly performed the preliminary section of the nerve, thereby placing the completeness and reality of the operation beyond dispute.

Two pieces, each about 1 centimetre in length, were removed from the left vagus nerve of this animal. One at the extreme upper limit of the nerve in the neck, and one at the extreme lower limit.

On the ninth day (ten inclusive of the day of operation) the piece of nerve which extended between the sites of the operation was removed.

From this piece the upper centimetre was excised, so as to present a new cross section distant 1 centimetre from the site of operation.

DEGENERATED VAGUS NERVE

Potential difference between points on the longitudinal surface (normally +) and the cross section

Point (1)*	o				
„ (2)	positive.	A deflection observed current too small to compensate, and, therefore, less than the unit of compensation ('00008 Daniell).			
„ (3)	„	„	„	„	„
„ (4)	„	„	„	„	„
„ (5)	„	„	„	„	„
„ (6)	„	„	„	„	„
„ (7)	„	„	„	„	„
„ (8)	The second cross section was negative. The deflection observed, but current again too small to compensate.				

A fresh section was now made at point (6), the subsequent examination revealed the same small difference between this point and the longitudinal surface as for the original cross section.

Resistance of piece 6 centimetres long measured = 30,100 ohms or 5,000 per centimetre. A fresh section was now made at point (5), subsequent examination revealed a similar state of things as before.

The nerve, 5 centimetres long, was now placed in tap water and left in this for 25 minutes, at the end of which time it was removed and dried in filter paper. Upon removal its altered appearance was noted. It was swollen and shorter, length 4.7 centimetres. It was rigid but pulpy in appearance, unlike the clean rigidity of a normal nerve after immersion in water.

Resistance measured was 51,400 ohms or 10,280 ohms per centimetre. *Potential differences* between longitudinal surface and upper cross section, and subsequently to lower cross section.

		Daniell				Daniell	
Point (1)	positive to upper section	...	'017424	to lower section	...	'012936	
„ (2)	„ „	...	'020064	„	...	'012936	
„ (3)	„ „	...	'015312	„	...	'012012	
„ (4)	„ „	...	'010296	„	...	'007920	
The lower cross section (5)		o					

* Point (1) as usual means point distant one centimetre.

A PIECE OF THE RIGHT, INTACT, VAGUS WAS NOW REMOVED, IN LENGTH
8 CENTIMETRES

The potential differences between points upon the longitudinal surface and the two cross sections were measured.

			Daniell			Daniell
Point (1)	positive to upper section	...	·000264	to lower section	...	·002376
" (2)	" "	...	·001320	"	...	·004092
" (3)	" "	...	·001320	"	...	·004092
" (4)	" "	...	·002376	"	...	·005016
" (5)	" "	...	·003036	"	...	·005808
" (6)	" "	...	·002640	"	...	·005280
" (7)	" "	...	·002376	"	...	·004356
" (8)	the lower cross section negative to upper ·000792 Daniell.					

A new cross section was now made at point (6). The potential difference between longitudinal surface and this (maximum) was ·007392 Daniell.

The resistance of nerve 6 centimetres long 69,300 ohms, or **11,550** per centimetre.

The lower centimetre was now excised, leaving the nerve 5 centimetres long.

This piece of nerve was then immersed in tap water and left in this for twenty-five minutes. Upon removal the nerve was slightly rigid, shortened to 4·7 centimetres, but did not appear swollen or pulpy.

The resistance was 96,300 ohms or **19,260** per centimetre.

The potential differences measured—

			Daniell			Daniell
Point (1)	positive to upper section	...	·013068	to lower section	...	·014114
" (2)	" "	...	·014520	"	...	·015182
" (3)	" "	...	·015048	"	...	·015182
" (4)	" "	...	·016632	"	...	·014256
" (5)	the lower cross section negative to the upper ·002904 Daniell.					

The examination of the second nerve was as the observations recorded shew, a repetition of the procedure of the examination of the first.

EXPERIMENT B

SCIATIC NERVE OF DOG. Bk. II, 102

On Thursday, July 26, 1900, Professor SHERRINGTON performed the preliminary operation upon this dog.

A piece 1 centimetre long (about) was removed from the extreme upper end of the right sciatic nerve trunk. The wound healed subsequently in the usual manner with no accident. On Tuesday, August 7, 1900, the dog was killed, that is on the twelfth of the days succeeding the day of the operation. The right sciatic nerve was removed, 4 centimetres being cut off below the operation section and thrown away, and a piece 5 centimetres being taken from below this for the purposes of the experiment.

(In all the succeeding statements of the potential differences, point (1), point (2), etc., means a point on the longitudinal surface distant 1 centimetre, 2 centimetres, etc., from the upper cross section).

- (a) The resistance of piece of 5 centimetres was 14,800 ohms, or **2,960** per centimetre. The potential differences between points on the longitudinal surface and the upper cross section were—

	Point (1) +	·000264	Daniell
	„ (2) +	·000264	
	„ (3) +	·000132	
	„ (4) +	·000132	
Other cross section	„ (5)* +	0	

The differences were therefore normal in direction, but small.

- (b) **The nerve now (ten minutes after its removal) was placed in tap water, and left in this for twenty minutes.** Upon removal it was dried in filter paper. It was now slightly rigid, and measured 5 centimetres. Its resistance was 20,200 ohms, or **4,040** ohms per centimetre.

	Point (1) +	·002772	volt potential differences to upper cross section.
	„ (2) +	·003960	„ „ „
	„ (3) +	·007128	„ „ „
	„ (4) +	·008712	„ „ „
Other cross section	„ (5)* +	·000132	„ „ „

- (c) *The nerve was now replaced in tap water, and left for a further twenty minutes in this.* The results of this, as of similar successive repetitions of the same manoeuvre, are given in the following table :—

(d), (e), (f), (g), (h), (i) in the table, represent repetitions of the same manoeuvre, and the subsequent observations made.

After this,* that is to say, four hours after the death of the dog, the nerve was placed in tap water, and examined after being in it twenty-four hours; the nerve was still tense, rigid, and swollen, and provided a demarcation current in the normal direction, as in the table line (j).

A similar observation was made the next day (k), the nerve having been now forty-eight hours in tap water—it was still rigid.

THE INTACT NERVE OF THE OTHER SIDE OF THE SAME ANIMAL

The left sciatic nerve was excised immediately after the first examination of the degenerated nerve was completed, and whilst the degenerated nerve lay in its first bath of tap water. This nerve was examined and then treated exactly in the same manner as the degenerated nerve was treated, the observations upon this nerve alternating with those upon the other, and so also the immersions in tap water. The main facts of this examination are given on following page.

Points marked thus * are in each case the second cross section

Immersion of Nerve in tap water	Length	RESISTANCE		Maximum Potential Difference found
		In ohms	In ohms per centimetre	
(a) Nerve at once	5	15,900	3,180	·0182
(b) After 20 min. in tap water ...	5	20,400	4,080	·0267
(c) Another 20 min. in tap water	5·2	23,100	4,440	·0269
(d) " " " ...	5·2	28,800	5,540	·0175
(e) " " " ...	5·2	39,800	7,650	·0151
(f) " " " ...	5·2	51,100	9,830	·0129
(g) " " " ...	5·2	54,900	10,540	·0166
(h) " " " ...	5·2	61,000	11,730	·0130
(i) " " " ...	5·2	88,700	17,060	·0174
The nerve was now placed in tap water for 24 hours, at the end of which time it was removed rigid, but not swollen, like the nerve of other side.				
(j) After 24 min. in tap water	—	307,500	60,000	·0045

Contrast these observations with the similar observations made upon the first nerve, the degenerated nerve. These are found in full detail in the table upon the next page.

EXPERIMENT B

DEGENERATED NERVE (SCIATIC OF DOG)

	Length in centimetres	RESISTANCE		POTENTIAL DIFFERENCES OF POINTS				
		In ohms	In ohms per centimetre	On longitudinal surface to the upper cross section (all points positive as in normal nerve)				Lower cross section to upper cross section
				Point (1)	Point (2)	Point (3)	Point (4)	Point (5)
(a) Nerve examined at once upon re- moval	5	14,800	2,960	.0003	.0003	.0001	.0001	0
(b) After having been placed for 20 min. in tap water	5	20,200	4,040	.0028	.0040	.0071	.0087	— .0001
(c) Another 20 min. in tap water ...	5.2	23,600	4,540	.0166	.0231	.0174	.0230	— .0031
(d) After another 20 min. in tap water	5.2	30,500	5,860	.0220	.0256	.0195	.0238	— .0001
(e) " " " " " "	5.2	35,300	6,790	.0209	.0256	.0209	.0227	— .0001
(f) " " " " " "	5.2	34,700	6,670	.0211	.0259	.0177	.0186	— .0001
(g) " " " " " "	5.2	45,800	8,800	.0132	.0221	.0127	.0157	— .0026
(h) " " " " " "	5.2	54,100	10,400	.0100	.0207	.0124	.0153	— .0037
(i) " " " " " "	5.2	59,600	11,460	.0091	.0178	.0107	.0116	— .0018
The nerve was now placed in tap water, left in this all night, and								
(j) Examined after 24 hours in tap water	5.1	192,500	37,740	.0001	.0046	.0055	.0055	+ .0039
Again left all night in tap water, and								
(k) Examined after 48 hours in tap water	5.1	442,500	86,760	0	.0005	.0003	.0003	+ .0002

THE ACTION OF SOLUTIONS OF ELECTROLYTES

PRELIMINARY EXPERIMENTS DECIDING THE CHOICE OF A CONVENIENT DURATION AND TEMPERATURE FOR IMMERSION OF THE NERVE

Three statements previously made are here repeated :—

- (1) That the 'core model' structure of nerve is a fact and demands at least the presence of—
 - (a) An external solution,
 - (b) A diffusion obstacle,
 - (c) An internal solution.
- (2) That probably the internal solution is the more concentrated of the two, that at least the two are certainly different.
- (3) That the injury current is the inevitable outcome of such a set of conditions.

These three statements are repeated because, with them in view, it is possible to intelligently follow all the modifications produced in the value of the injury current of nerve by the action of solutions of electrolytes. Thus granted that the value of this current (more strictly of the P.D.) depends upon the ratio existing between the concentrations of the two solutions, it at once follows that a replacement of the outer solution by a still more dilute solution should increase this value, and that replacement by a more concentrated solution should diminish it. It will be seen that this apparently antagonistic action between more dilute and more concentrated solutions has been found in the case of solutions of several electrolytes, it may be said in every such case in which it has been sought.

There is, in fact, however, no real antagonism between the action of dilute and concentrated solutions of electrolytes. There is an apparent antagonism, because an arbitrary standard is chosen for comparison, namely, the concentration of the external solution already present upon the nerve when removed from the body. All appearance of paradoxical action is at once removed by the acceptance of water as a zero of concentration.

The action of water was studied in the last section ; it is now our intention to study this action diminished by the addition of electrolytes to the water used, and it will be seen that the diminution increases with the quantity of electrolytes which is added.

Before doing so, however, it is necessary to examine the limits within which such a study can be pursued, limits which have already made their appearance as modifying the action of water. The first and most important of which is that set by the nature of the diffusion obstacle separating the external and internal solution.

It is not claimed for this 'membrane' that it is an absolute barrier to processes of diffusion between the two solutions. Such a claim would place it in a unique position amongst all the other limiting cell surfaces of the body as a strictly defined 'semi-permeable membrane;' would place it in fact, if logically maintained, in a unique position amongst the membranes studied by the physicists and called by them 'semi-permeable.' This membrane was called into theoretical being by necessities which arose in explanation of the polarization phenomena, and, once postulated, its existence was found, from general analogy and from other circumstances, to be not only not contra-indicated but even to be confirmed. Even if it be admitted, and it is not, that the necessities of the polarization phenomena form, after this subsequent examination, its only claim for existence; still, it must be granted that these necessities are far from demanding a strictly semi-permeable membrane. All that is required by them is that the membrane shall be a 'partial' barrier to the movement of dissolved electrolytes, and shall so give rise to the accumulation of charged particles on its surfaces, particles left behind by their fellows which have successfully traversed it, and so conducted the electrical current. In short, neither theoretical necessity nor the teachings of physiological analogy point to the presence of anything but an imperfect obstacle to processes of diffusion between the solutions of the nerve.

Granted that this is so, and that as observed in the action of water (see page 275) the effect of immersing a nerve in a given solution is not merely to replace with this the external solution of the nerve, but also by penetration of the diffusion obstacle to modify the internal solution; then here certainly we have a reason for accepting the formerly rejected standard of concentration, namely, that of the 'isotonic solution,' which is removed with the nerve from the body.

Granted that an immersion affects both the solutions of the nerve, then immersion in a dilute solution

- (1) dilutes the external solution and so increases the injury current,
- (2) dilutes the internal solution and so diminishes the injury current;

whereas immersion in a concentrated solution

- (1) concentrates the external solution and so diminishes the injury current,
- (2) concentrates the internal solution and so increases the injury current.

It is fortunate that the second effect must in both cases be completed later than the first, and that so an opportunity is left for the study of the first: even if it is not as perfect as a physicist, with the advantages of determining his own conditions, would choose for the examination of the differences of potential caused by diffusion into contrasted solutions. To make the most of the opportunity it is necessary to accept

the indication of preliminary experiments in choosing the duration of time most suitable for the immersion of the nerve in the solutions used. The shorter the time the less will be the undesired modification of the internal solution of the nerve. It is thus necessary to fix a time not too short for the best replacement attainable of the outer solution, and yet as short as possible so as to ward off this antagonistic modification.

EXPERIMENT I

SCIATIC NERVE OF CAT

A piece of Nerve 5 centimetres long

Value of injury 'current' upon removal	...	15	$\times 10^{-3}$	Daniell
After an immersion of 1 hour and 40 minutes in				
9 per cent. NaCl solution	1	"	"
After a subsequent immersion of 15 minutes in				
tap water	11	"	"
<i>After a further immersion of 30 minutes in tap water</i>				
<i>(45 minutes in all)</i>	22	"	"

The solution of NaCl used was maintained at 17° C. After each immersion the nerve was dried in filter paper. The nerve was examined several times during its prolonged immersion in the concentrated NaCl solution, after the first twenty-five minutes, a second twenty-five minutes, a third twenty-five minutes, and then ten minutes; on each occasion the value of the injury potential difference (longitudinal surface to upper cross section) was found equal to .001 Daniell.

EXPERIMENT II

SCIATIC NERVE OF CAT

A piece of Nerve 5 centimetres long

Value of injury 'current' upon removal	...	17	$\times 10^{-3}$	Daniell
After 5 minutes in .45 per cent. NaCl solution		24	"	"
After 45 minutes (in all) in .45	" "	3	"	"
<i>After a subsequent immersion of 20 minutes in tap</i>				
<i>water</i>	0	"	"

The data from these experiments give point to the remarks just made. One of these experiments shews the effect of immersion in a concentrated solution ten times the strength of the isotonic solution; the other the effect of immersion in a dilute solution one-half the strength of the isotonic solution.

In both cases, prolonged immersion in the solution has reduced the original value of the potential difference down to a small fraction. The reduction in the case of the nerve immersed in the dilute solution is irreparable by the subsequent immersion

of the nerve in water. On the other hand, an immersion in water succeeds in bringing back to and beyond its original value the potential difference from the nerve which has been lying in the highly-concentrated solution.

The inference to be drawn from such facts seems an obvious one. The 'internal solution' of the nerve which had been immersed in the concentrated solution had become more concentrated, that of the nerve which had been immersed in the dilute solution had become more dilute; the distinction between their final states is revealed by a subsequent extreme dilution of their 'external solutions' (action of tap water) and subsequent testing.

EXPERIMENT III

SCIATIC NERVE OF CAT

A piece of Nerve 5 centimetres long

Nerve immediately after removal	17.0	$\times 10^{-3}$	Daniell
After 25 minutes in 1.8 per cent. NaCl solution	...		6.6	"	"
After a further 30 minutes in 1.8 per cent. NaCl solution			3.6	"	"
After a further 15 minutes	"	"	2.9	"	"
After a further 15 minutes	"	"	2.6	"	"
After a further 15 minutes	"	"	2.6	"	"

EXPERIMENT IV

SCIATIC NERVE OF CAT

A piece of Nerve 5 centimetres long

Nerve immediately after removal	14.8	$\times 10^{-3}$	Daniell
After 25 minutes in .9 per cent. NaCl solution	...		11.6	"	"
After a further 25 minutes in .9 per cent. NaCl solution			9.6	"	"
After a further 25 minutes	"	"	6.9	"	"
After a further 25 minutes	"	"	6.5	"	"

EXPERIMENT V

SCIATIC NERVE OF CAT

A piece of Nerve 5 centimetres long

Nerve immediately after removal	13.5	$\times 10^{-3}$	Daniell
After 25 minutes in .6 per cent. NaCl solution	...		14.8	"	"
After a further 25 minutes in .6 per cent. NaCl solution			14.5	"	"
After a further 25 minutes	"	"	8.6	"	"
After a further 25 minutes	"	"	5.0	"	"
After a further 25 minutes	"	"	1.6	"	"

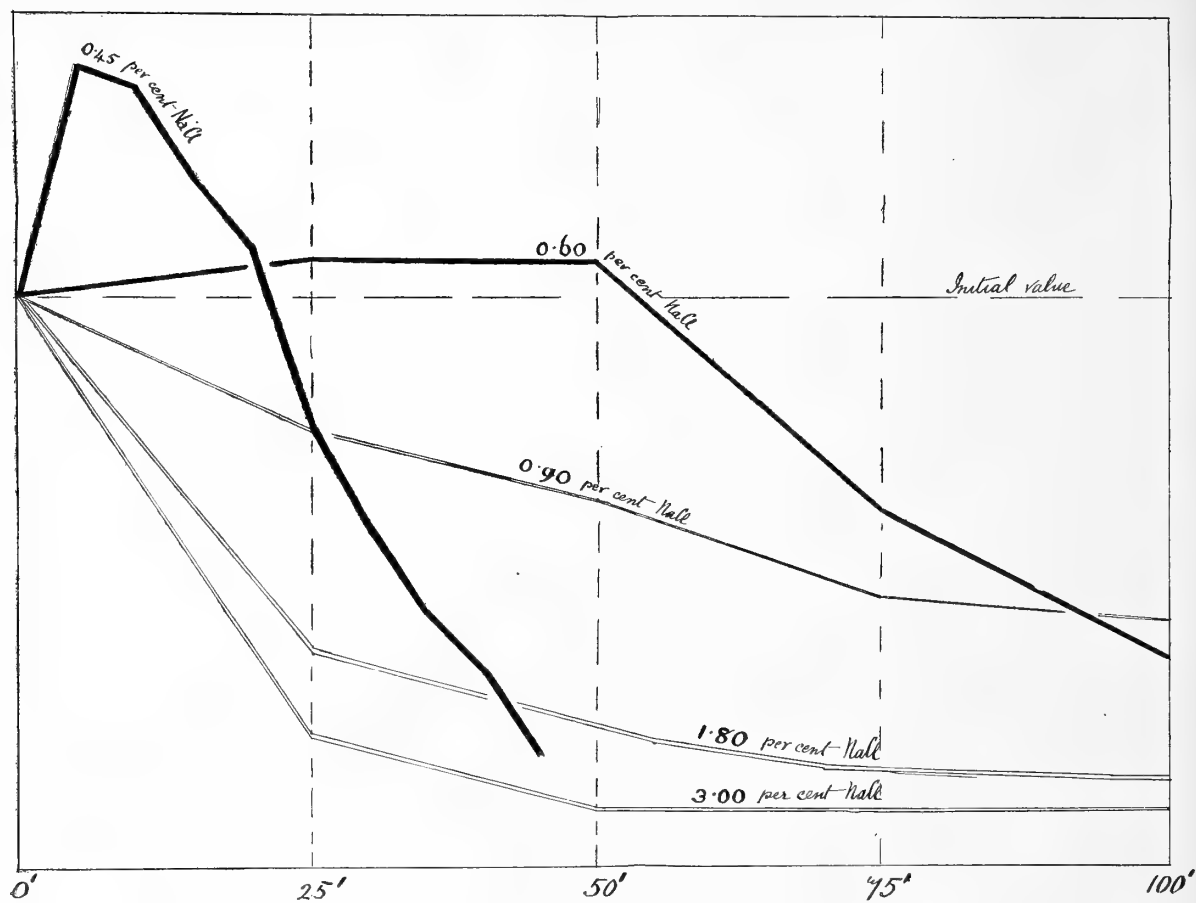


FIG. A

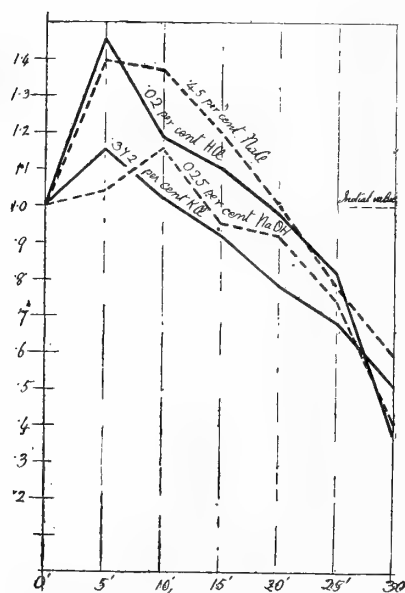


FIG. B

FIG. A includes curves drawn from Experiments IIa, III, IV, and V.

FIG. B includes curves drawn from Experiments IIa, VI, VII, and VIII.

In these experiments all the solutions used were dilute. The curves represent the action of dilute solutions of different electrolytes. In the previous figure, A, the curves represent the action of the same electrolyte at different concentrations.

The curves in figure A have been obtained in the following manner. In each experiment the original potential difference determined immediately after removal of the nerve from the body is treated as unity, and all the modifications obtained by the effect of immersion in the solution are expressed in terms of this.

Thus taking the data from the three experiments—

EXPERIMENT III 1·8 % NaCl		EXPERIMENT IV ·9 % NaCl		EXPERIMENT V ·6 % NaCl	
Original	1·000	Original	1·000	Original	1·000
In 25 min.	0·388	In 25 min.	0·786	In 25 min.	1·060
In 55 „	0·211	In 50 „	0·643	In 50 „	1·059
In 70 „	0·170	In 75 „	0·482	In 75 „	0·635
In 85 „	0·153	In 100 „	0·429	In 100 „	0·365
In 100 „	0·153			In 125 „	0·115

The curves serve to shew the orderly fashion in which solutions of different concentration modify the injury current, or a better form of statement, perhaps, modify the decline of the injury current. The more concentrated the solution, the more rapid and complete the apparent decline; the more dilute the less rapid, so that the most dilute solutions temporarily enhance the value of the injury current.

As will be seen from the curves, this statement is unexceptionable when the early portion of the curves is considered. In this portion it is realized that the concentration limits—of pure water on one side and saturated solution on the other—are associated with the extremes of effect produced, increase of the injury current on one side and elimination on the other. Between the limits of concentration the effect is dependent upon the concentration, the graduated effect being only adequately described by taking the effect of one of these limits of concentration—pure water—as the maximum, and describing the remainder as the effects of solutions more concentrated than this.

It is only when the later portions of the curves is considered that the isotonic solution demands attention as the solution in which the injury current normally declines; separating more concentrated solutions which continue to follow the course described throughout the whole time of the experiment from less concentrated solutions, which adopt a new and paradoxical course.

The later action of these less concentrated solutions is quite characteristic, and in direct opposition to their earlier effects. For they are seen secondarily to cause a

more rapid and more complete decline of the injury current the less their concentration. This later decline is advisedly called 'more complete' since the previous experiments have shewn it to be irreparable, and quite unlike the decline observed after immersion in more concentrated solutions, which can be recovered from by a subsequent immersion in water.

Such a comparison of the effects of solutions of different concentrations is also fully justified by an examination of the numerical values.

Let us, as a test of this statement, assume that during the first twenty-five minutes of immersion the 'internal solution' of the nerve is not greatly modified, then the modifications in the value of the potential difference should be comparatively simple, and should, crudely, vary inversely with the concentration of the solution used to replace the external solution.

$$\text{Experiment III—} \cdot 388 = \frac{x}{1\cdot8} \quad \therefore x = \cdot 6984$$

$$\text{Experiment IV—} \cdot 786 = \frac{x}{\cdot 9} \quad \therefore x = \cdot 7074$$

$$\text{Experiment V—} 1\cdot 060 = \frac{x}{\cdot 6} \quad \therefore x = \cdot 6360$$

The value x obtained upon the assumption that the values vary inversely with the concentration of the experimental solution made use of, are seen to be approximately similar enough to justify the assumption within these limits of concentration. It is even possible to extend these limits widely, and still the facts remain fairly closely within the limits of this relation.

Thus, from experiments performed, including those given above, the following table was constructed and published in a preliminary communication* :—

Strength of Experimental Solution of NaCl					Value of x as calculated above
0·6 grammes per cent.	·6360
0·75	"	"	·6908
0·9	"	"	·7074
1·8	"	"	·6984
3·0	"	"	·7110
6·0	"	"	·6420
9·0	"	"	·5580

* *Proceedings Royal Society*, 67, 321

The limits within which the relation is most true is seen to be from .75 per cent. to 3.0 per cent., within which it may be said that the relation is actually that here stated. But when solutions are used much below the strength of the isotonic solution, the limit of even approximate truth is rapidly reached and passed. The reason, or certainly one of the reasons, of this fact is not far to seek, and is clearly brought out by the details of the following experiment, and by the curve drawn from them and placed in the diagram given (.45 per cent. curve):—

EXPERIMENT II_A

(AN EXPANSION OF THE DETAILS OF EXPERIMENT II)

	POTENTIAL DIFFERENCES	
	× 10 ⁻³ Daniell	In terms of the original as unity
Upon removal	17.1	1.00
(5) After 5 min. in .45 NaCl	24.0	1.40
(10) Another 5 min. in .45 NaCl	23.9	1.37
(15) " " " " " "	20.6	1.20
(20) " " " " " "	18.7	1.09
(25) " " " " " "	13.6	0.79
(30) " " " " " "	10.3	0.60
(35) " " " " " "	7.9	0.46
(40) " " " " " "	6.1	0.35
(45) " " " " " "	3.4	0.20

In this case the value of the potential difference after 25 minutes in the solution is 0.79.

$$.79 = \frac{x}{.45} \quad \dots \quad x = .35$$

The value of x is just half of that which would be found if the value of the potential difference varied inversely with the concentration of the solution. Nor is there, as yet, any reason to modify the statement of this temporarily assumed relationship; for one fact, and an unavoidable fact, spoils the value of the experiment from this point of view. The theoretical value required by the relation, 1.50, was almost attained in the experiment, but this was in the first five minutes, when the value rose to 1.40. After this the value has steadily fallen until in forty minutes it has reached a value as low as that reached by the nerve in Experiment V (.6 per cent. NaCl) in a time four times as great.

Immersion in a dilute solution increases the value of the 'injury current' as long as the immersion leaves the concentration of the 'internal solution' of the nerve fairly intact: but prolonged immersion rapidly dilutes this internal solution and diminishes the value of the injury current, and the more dilute the experimental solution, the more rapid and the more final is this diminution.

Such considerations as these may serve to justify the course adopted in the experiments of the next section, for the results of which a real quantitative value is claimed. In these experiments the time of immersion adopted is of five minutes duration, and no attention is paid to modifications produced by a more prolonged immersion than this.

Since in this section alone the effects of prolonged immersion are studied, it seems better to include here a few experiments which completely shew the similarity in action upon the injury current of dilute solutions of the following electrolytes:—

Sodium chloride. (Already given in Experiment II_A).

Caustic soda.

Hydrochloric acid.

Potassium chloride.

EXPERIMENT VI SCIATIC NERVE OF CAT

Piece of Nerve 5 centimetres long. Experimental solution used '372 grammes per cent. KCl.

	POTENTIAL DIFFERENCES	
	$\times 10^{-3}$ Daniell	In terms of the original value as unity
Nerve upon removal	19.5	1.00
(5) After 5 min. in '372 per cent. KCl. ...	22.4	1.15
(10) Another 5 min. in '372 per cent. KCl. ...	20.1	1.02
(15) " " " " ...	18.0	0.92
(20) " " " " ...	15.3	0.78
(25) " " " " ...	13.2	0.68
(30) " " " " ...	10.0	0.51
(35) " " " " ...	9.2	0.47
(40) " " " " ...	6.6	0.34
(45) " " " " ...	5.8	0.30
(50) " " " " ...	4.8	0.24
After 10 min. in tap water	4.0	0.20

This nerve was removed immediately after the death of the animal. The second sciatic nerve was, as a contrast (see below), removed one-and-a-half hours after death, and similarly examined.

THE SECOND NERVE

	Potential Differences
Nerve upon removal	9.8×10^{-3} Daniell
After 5 min. in '372 per cent. KCl. ...	13.7
(5) Another 5 min. in '372 per cent. KCl. ...	15.0
(10) " " " " ...	19.5
(15) " " " " ...	17.4
(20) " " " " ...	17.0
(25) " " " " ...	14.2
(30) " " " " ...	10.5

EXPERIMENT VII

SCIATIC NERVE OF CAT

(a) Piece of Nerve 5 centimetres long. Experimental solution used for immersion of nerve '025 of NaOH. Nerve removed immediately after the death of the animal.

	POTENTIAL DIFFERENCES	
	$\times 10^{-3}$ Daniell	In terms of the original value as unity
Nerve upon removal	23.0	1.00
(5) After 5 min. in '025 per cent. NaOH ...	23.9	1.04
(10) Another 5 min. in '025 per cent. NaOH ...	26.7	1.16
(15) " " " " " ...	22.1	0.96
(20) " " " " " ...	21.2	0.92
(25) " " " " " ...	17.0	0.74
(30) " " " " " ...	9.2	0.40
(35) " " " " " ...	6.8	0.3†

(b) The other nerve of the same animal removed one hour after death of the animal—

	POTENTIAL DIFFERENCES		
	$\times 10^{-3}$ Daniell	In terms of the original value as unity	In terms of the original value of the first nerve as unity
Nerve upon removal	14.4	1.00	0.62
(5) After 5 min. in '025 per cent. NaOH ...	23.7	1.66	1.04
(10) Another 5 min. in '025 per cent. NaOH ...	24.6	1.70	1.07
(15) " " " " " ...	22.1	1.54	0.96
(20) " " " " " ...	21.2	1.47	0.92
(25) " " " " " ...	16.1	1.12	0.70
(30) " " " " " ...	9.9	0.68	0.43

EXPERIMENT VIII

SCIATIC NERVE OF CAT

(a) Piece of Nerve 5 centimetres long. Experimental solution used for immersion of the nerve '02 per cent. HCl. First nerve examined immediately after death of the animal—

	POTENTIAL DIFFERENCES	
	$\times 10^{-3}$ Daniell	In terms of the original value as unity
Nerve on removal	17.7	1.00
(5) After 5 min. in '02 per cent. HCl ...	25.9	1.46
(10) Another 5 min. in '02 per cent HCl ...	21.1	1.19
(15) " " " " ...	19.5	1.10
(20) " " " " ...	17.2	0.97
(25) " " " " ...	14.5	0.82
(30) " " " " ...	6.9	0.39

(b) The second nerve removed one hour after death—

	POTENTIAL DIFFERENCES		
	$\times 10^{-3}$ Daniell	In terms of the original value as unity	In terms of the original value of the first nerve as unity
Nerve on removal	12.4	1.00	0.70
(5) After 5 min. in '02 per cent. HCl ...	25.9	2.09	1.46
(10) Another 5 min. in '02 per cent. HCl ...	22.4	1.80	1.26
(15) " " " " ...	19.8	1.59	1.12
(20) " " " " ...	18.5	1.49	1.04
(25) " " " " ...	13.2	1.06	0.75
(30) " " " " ...	5.3	0.43	0.30

Modification in the injury 'current' (P.D.) produced by immersion of the nerve in dilute solutions of electrolytes.

			EXPERIMENT II (A)	EXPERIMENT VII	EXPERIMENT VI	EXPERIMENT VIII
			.45 per cent. NaCl	.372 per cent. KCl	.025 per cent. NaOH	.02 per cent. HCl
Nerve at once	1.00	1.00	1.00	1.00
After 5 min. immersion	1.40	1.15	1.04	1.46
„ 10 „ „	1.37	1.02	1.16	1.19
„ 15 „ „	1.20	0.92	0.96	1.10
„ 20 „ „	1.09	0.78	0.92	0.97
„ 25 „ „	0.79	0.68	0.74	0.82
„ 30 „ „	0.60	0.51	0.40	0.39

From the curves in figure B, or from the numbers in the table, it is possible to appreciate the close similarity in effects of all these dilute solutions of electrolytes. This is the more remarkable if one considers that there is every reason to suppose that a better selection of corresponding concentrations of these different electrolytes would have led to a closer correspondence still. The curves are very similar, and are quite unlike the curves obtained from concentrated solutions as is seen from the curves of figure A. It is also obvious that they form evidence strengthening the conclusion already arrived at, that real attempts to quantitatively estimate the modification produced by immersion in a solution of electrolytes had better be limited to a study of the results of the first five minutes' immersion.

The details of these experiments bears witness also to another very interesting fact which is brought out by the contrasted examination of nerves removed at different periods after the death of the animal.

For a very considerable interval after the death of the animal the nerves are practically unaltered by the changes which immediately follow death, as far at least as the characteristics of structure are concerned which give rise to the injury current, except in one particular.

The outer solution of the nerve is synonymous with the lymph of the tissue; and the nerves examined (the sciatic nerves) lie, while in the body, imbedded in great muscular masses. From these muscles carbonic acid and other disintegration products are continually being cast off, which, during life, are removed by the circulating blood,

but after death accumulate locally in the lymph of the part in which they are formed. The outer solution of the nerve is therefore being gradually altered after the death of the animal by the concentration in it of electrolytes derived in the first place from the surrounding muscles.* Such a concentration of the outer solution leads to a diminution in the injury current, and nerves removed from the body even five minutes after death exhibit a diminution in the demonstrable injury current, and from this cause.

When the nerves are immersed for a short time in a solution which successfully replaces the 'external solution' originally present, they are then in a more standard condition for comparison than when the variable 'external solution' remains. A comparison under such circumstances gives more reliable information as to the condition of the other factors necessary to the manifestation of the injury current, *e.g.*, the tubular membrane and the enclosed 'internal solution.'

DATA FROM EXPERIMENTS VII AND VIII

EXPERIMENT VII. '025 per cent. NaOH		EXPERIMENT VIII. '02 per cent. HCl	
First nerve removed at once	Second nerve removed in 1 hour	First nerve removed at once	Second nerve removed in 1 hour
Potential Difference 23'0	14'4	17'7	12'4
After successive immersions in their respective solutions.	(1) 23'9	25'9	25'9
	(2) 26'7	21'1	22'4
	(3) 22'1	19'5	19'8
	(4) 21'2	17'2	18'5
	(5) 17'0	14'5	13'2
	(6) 9'2	6'9	5'3

In each of these experiments it is obvious that whereas a great difference exists between the nerve removed at once and that removed an hour later, when first examined: *yet the first immersion has in either case placed the two nerves upon an absolute equality*, which is maintained throughout their subsequent history.

It is worthy of note that the first beneficent effect of immersion is produced alike by dilute solutions of acid or of alkali, the following experiment will shew, too, that there is no particular virtue in the fact that these solutions are dilute.

* See Waller, *Animal Electricity*, p. 56

EXPERIMENT IX

SCIATIC NERVE OF CAT

Piece of Nerve 5 centimetres long. Experimental solution used : '25 per cent. NaOH.

FIRST NERVE removed at once						SECOND NERVE removed in one hour
Potential difference of the nerve upon removal	...	20.1	$\times 10^{-3}$	D.		10.6×10^{-3} D.
(5) After 5 minutes in '25 per cent. NaOH	...	9.0		"		8.2 "
(10) Another	"	"	"	...	3.4 "	5.2 "
(15)	"	"	"	...	2.1 "	2.1 "
(20)	"	"	"	...	1.6 "	1.8 "

In this experiment the same fact is seen elicited by a solution which has the typical effect of a concentrated solution. Nor is this revelation of the still pristine vigour of the nerve limited to the action of acids and alkalies, it may be observed more or less completely in the case of the action of any solution of electrolytes. A reference to the data of Experiment VI will show the same influence at work, as revealed (at a longer interval after death) by the solution of a neutral salt (KCl). In the next section the influence of this factor is seen in the data obtained from every solution of electrolytes used: the preliminary difference is there seen as the result of a stay of only five minutes longer in the body, and its removal is seen as a consequence of immersion in many different solutions.

In all the experiments previously quoted, the experimental solutions into which the nerve was placed have been of the temperature of the room. In the succeeding section care has been taken that in every case the solution used should be at the same temperature, 18°C ., and that this should be maintained constant. In an examination of a process presumably dependent upon a diffusion process, the precaution of maintaining a constant temperature is an obvious necessity, the rate of diffusion being notably influenced by temperature. The choice of a standard temperature is more or less a matter of convenience, and the most convenient, from the point of view of physical measurements, is the temperature chosen. This standard temperature was not, however, chosen at once, since in the case of mammalian nerve certain other considerations are of value. The nerve removed rapidly from the animal is already at a temperature of 38°C . approximately, therefore scruples which dictate a study of, what is called, nerve in a normal condition point to the selection of this temperature for the examination of the nerve. One scruple of this kind, more definite than the remainder, is strongly in favour of such a course, namely, that which is affected by the condition of the myelin sheath.

A temperature of 38°C . is not, however, so easily maintained as a temperature of 18°C ., it is also a temperature at which the rate of decline of the injury current is very great, as would the rate of any process of diffusion also be. In an attempt to study the quantitative effect of immersion in various solutions, this declining value of the original phenomenon has to be borne in mind as carefully as in the case of any of the other measurements undertaken previously. Nor can this source of error be as easily dealt with as in the case of the examination of the distribution potential (see previous section on 'current of injury'). In that case the error was eliminated by taking two sets of measurements in a regular order. In this case no such method is applicable, since, as has been clearly stated, even a short immersion in any solution (which is not the 'isotonic' one) leaves a fractional, but still important, effect behind it upon the concentration of the 'internal' as well as of the 'external' solution of the nerve. At first this point was not as clearly recognized as now, and experiments were made by the author in which immersions in the experimental solution were alternated with immersions in an isotonic solution: such experiments, although of some interest, have no quantitative importance, and have the disadvantage of appearing to correct an error which they largely leave unmodified, or only modify it in a new and undesirable fashion.

The decline of the injury current is, therefore, an important consideration, and is most satisfactorily dealt with by immersions in solutions at a temperature unfavourable to its marked occurrence.

Data have already been given* from experiments in which the influence of temperature was studied, the data of the following experiment will serve here as an illustration:—

EXPERIMENT X

SCIATIC NERVE OF CAT

(a) First nerve, removed immediately after death:—

Potential difference at once	13.3×10^{-3}	Daniell
After 25 minutes in .75 per cent. NaCl solution at 17°C .				12.5	„

(b) Second nerve, removed immediately after death:—

Potential difference at once	13.3	„
After 25 minutes in .75 per cent. NaCl solution at 38°C .				8.2	„
After a subsequent 5 minutes in .75 per cent. at 17°C .				8.1	„

In this experiment the two nerves were fortunately in the same original condition, and the degree of modification by immersion in the same solution at different temperatures is clearly seen. At a temperature of 38°C . the decline in the injury current is 39 per cent. of the original, at 17°C . the decline is only 6 per cent. in the same interval of time.

* J. S. Macdonald, *Preliminary Communication, Proc. Roy. Soc.*, 67, 321.

There is, however, another fact to consider, namely, that if at any time the temperature of the nerve could be suddenly changed from 38°C. to 17°C. , the value of the injury current (as determined by a diffusion process) would be found to be quite different, without there being any intervening differential rate of decline to consider. In view of such consideration it will be seen that in the second part of the experiment the temperature of the nerve, which had been for twenty-five minutes at 38°C. , was changed to 17°C. without producing any alteration in the low value due to more rapid decline through the preceding twenty-five minutes. The result of this secondary modification in this experiment is also of interest from another point of view. A differential modification of the temperature of the 'internal' and 'external' solutions of the nerve might be of importance. Taking the main characteristic of the physical structure of nerve to be the separation of its solutions by tubular membranes, such a characteristic might conceivably be a factor determining that the temperature of the 'external solution' should be more readily capable of modification than that of the 'internal solution.' Such a differential modification would alone account for great modification of the injury current, and is probably accountable for differences observed in the nerve injury current at very different temperatures of the air. It is satisfactory, therefore, to note that the modification produced in five minutes must have, in view of the result obtained, equally affected all the factors in the production of the injury current. For had the cooling affected only the external solution, the injury current would have been increased. Immersion in a solution at a temperature of 18°C. is therefore convenient, and also five minutes is an adequate time in which to bring the whole nerve approximately to this temperature.

QUANTITATIVE COMPARISONS

EXPERIMENTAL PROCEDURE

Non-polarizable electrodes, having been prepared, were placed in position 2.5 centimetres apart.

A cat having been killed, a piece of one sciatic nerve, 5 centimetres long, was immediately removed.

The potential difference was measured between the upper cross section and a point on the longitudinal surface 2.5 centimetres distant.

The nerve was then immersed in the solution which had been previously prepared and brought to a temperature of 18° C. The nerve was left in this for five minutes exactly timed. During the immersion the vessel containing the solution was frequently shaken. The quantity of the solution used was always the same, 200 cc.

At the end of five minutes the nerve was removed. This was accomplished by seizing its lower end, which is always easily identified, in fine pointed forceps.

The nerve was placed upon a sheet of filter paper, which was then folded upon it. Complete drying was obtained by rolling the nerve and longitudinally compressing it between finger and thumb in the fold of filter paper. This drying operation was repeated three times, a dry place in the filter paper being used each time.

The potential difference was then again measured.

When the second sciatic nerve of the same animal was used, a routine method was followed. As soon as the first nerve had been placed in the prepared solution the second was immediately removed, examined, and placed in a separate vessel of solution just as the time approached for the final examination of the first nerve. In this the examinations and immersions of the two nerves were made to alternate, and consequently only a small interval of time elapsed between the removal of the two nerves. Even this slight difference was not, however, without its consequence (for reasons, see previous section), and accordingly in all the experiments quoted such nerves are always marked '(2)' to distinguish them from the nerves prepared first, which are marked '(1).'

The confidence placed in a single examination of the potential difference between the upper cross section and the mid-point of the nerve was the outcome of the experiments performed in the first section of this paper. In the curves of that section it will be noticed that the main variations in the value of the potential difference take place in the first two centimetres, and are unimportant at a greater distance.

The solutions were made up with pure chemicals dissolved in distilled water.

Great care was taken to insulate all the conducting paths used in the measurements taken, with the intention of making the results obtained as reliable as possible. For the same reason the drying of the nerve was always complete, and the attempt at drying the nerve after immersion in a solution was always carried to a point when the nerve left no further visible trace of moisture upon the filter paper used.

The measurement of potential difference was always accomplished by the usual compensation method. A large (quart) Daniell cell being carefully made up each morning for experiments carried out in the afternoon.

All the figures given are the result of quite separate experiments, in each of which an initial value and a final value for the potential difference having been obtained, before and after immersion, the nerve was thrown away.

In all cases the nerves used were, as stated above, the sciatic nerves of cats.

TABLE OF EXPERIMENTS

	CONCENTRATION OF THE SOLUTION USED		POTENTIAL DIFFERENCE			Number of Experiment
	(1) In grammes per cent.	(2) In gram-molecular per litre	(1) Initial Value $\times 10^{-3}$ Daniell	(2) Final Value $\times 10^{-3}$ Daniell	(3) Final value in terms of the initial value as unity	
Solutions of Hydro- chloric acid HCl	·2	$\frac{1}{18.2}$	13.46	4.22	0.31	Experiment I (I)
	·1	$\frac{1}{36.3}$	13.99	8.71	0.62	„ II (I)
	·025	$\frac{1}{145.2}$	15.84	19.27	1.21	„ III (I)
	·0125	$\frac{1}{290.4}$	18.48	28.25	1.51	„ IV (I)
Ammonium Chloride NH ₄ Cl	5.35	1	14.52	0.66	·045	Experiment V (I)
	5.35	1	18.74	1.92	·102	„ VI (I)
	2.67	$\frac{1}{2}$	15.05	4.89	·325	„ VII (2)
	2.67	$\frac{1}{2}$	17.16	5.68	·330	„ VIII (2)
	1.33	$\frac{1}{4}$	14.52	9.50	·654	„ IX (I)
	0.67	$\frac{1}{8}$	13.73	12.14	·884	„ X (2)

TABLE OF EXPERIMENTS—*continued*

	CONCENTRATION OF THE SOLUTION USED		POTENTIAL DIFFERENCE			Number of Experiment
	(1) In grammes per cent.	(2) In gram-molecular per litre	(1) Initial Value x 10 ⁻³ Daniell	(2) Final Value x 10 ⁻³ Daniell	(3) Final value in terms of the initial value as unity	
Lithium Chloride LiCl	4.25	1	19.67	5.28	0.268	Experiment XI (1)
	2.12	$\frac{1}{2}$	20.72	12.28	0.593	„ XII (1)
	1.06	$\frac{1}{4}$	19.11	17.42	0.911	„ XIII (2)
	0.53	$\frac{1}{8}$	15.58	19.01	1.220	„ XIV (2)
Calcium Chloride $\frac{1}{2}$ (CaCl ₂)	5.55	1	19.44	6.88	.354	Experiment XV (1)
	2.77	$\frac{1}{2}$	19.27	12.49	.648	„ XVI (1)
	1.39	$\frac{1}{4}$	21.00	18.40	.876	„ XVII (1)
Barium Chloride $\frac{1}{2}$ (BaCl ₂)	10.4*	1	17.69	4.36	.245	Experiment XVIII (1)
	5.2	$\frac{1}{2}$	13.60	6.73	.495	„ XIX (2)
	2.6	$\frac{1}{4}$	18.22	12.67	.695	„ XX (1)
	1.3	$\frac{1}{8}$	13.20	12.54	.950	„ XXI (2)
Sodium Chloride NaCl.	5.85	1	20.59	2.77	.135	Experiment XXII (1)
	2.92	$\frac{1}{2}$	17.16	5.28	.308	„ XXIII (1)
	2.92	$\frac{1}{2}$	17.42	5.03	.289	„ XXIV (1)
	1.46	$\frac{1}{4}$	20.59	13.73	.666	„ XXV (1)
	1.46	$\frac{1}{4}$	16.63	9.77	.588	„ XXVI (2)
	0.73	$\frac{1}{8}$	16.63	15.04	.904	„ XXVII (1)
Potassium Chloride + KCl.	7.45	1	14.52	1.59	.109	(1)
	3.72	$\frac{1}{2}$	24.29	9.90	.407	(1)
	1.86	$\frac{1}{4}$	14.78	9.50	.640	(1)

* Including Water of Crystallization, total weight, 12.2 grammes per cent.

† Potassium Chloride Solutions will be found later treated as a special case, the experiment given here are taken from experiments given later.

In this table are collected the results of twenty-eight experiments in which should be found data sufficient to test any presumed relationship existing between the effects of immersing the nerve in different concentrations of the same electrolyte, and also of different electrolytes. The immersions have been in solutions of seven electrolytes, all chlorides—

Hydrochloric acid,
Ammonium chloride,
Lithium chloride,
Calcium chloride,
Barium chloride,
Sodium chloride,
Potassium chloride.

It is of interest to commence with an examination of the relationship which we preliminarily tested, namely, a variation inversely as the concentration: and also with solutions of hydrochloric acid, for in their case the real relationship, as will subsequently be seen, is most obvious and convincing.

The concentration of hydrochloric acid made use of are evidently capable of producing a wide range of effects. Solutions of $\cdot 025$ and $\cdot 013$ grammes per cent. produce, and in the right relative degree, the typical effects of dilute solutions, increasing the injury current. Solutions of $\cdot 1$ and $\cdot 2$ per cent. produce the typical effects of concentrated solutions, diminishing the injury current, and also in the right relative degree. If in addition to this conformity to the general statement, they also conform to the assumed arithmetical relation; then the value of 'x' in each of the following cases should be the same:—

Experiment I—	$x = \cdot 2$	$\times \cdot 31 = \cdot 62$
Experiment II—	$x = \cdot 1$	$\times \cdot 62 = \cdot 62$
Experiment III—	$x = \cdot 025$	$\times 1\cdot 21 = \cdot 30$
Experiment IV—	$x = \cdot 0125$	$\times 1\cdot 51 = \cdot 19$

In the first two of these experiments the value for 'x' is the same, and therefore, for these two cases, and presumably for intervening examples, it might be said that the potential difference varies inversely with the strength of the experimental solution. But when we turn to the remaining experiments (III and IV) we find that such a relation no longer exists, as is also the case with similar solutions of NaCl, *i.e.*, those which also cause an increase in the value of the injury current.

Faced with such a fact, it is natural to enquire the reason which induced us to seek for, and to appreciate when found, the existence of this inverse relationship of the value of the injury current to that of the experimental solution. The reason

undoubtedly was based upon the opinion that the injury current was caused by an inequality in the 'internal' and 'external' solutions of the nerve, and might therefore be expected to depend upon the ratio existing between them, *e.g.* :—

$$\frac{\text{Internal solution}}{\text{External solution}}$$

But such an expectation is not based upon a knowledge of the value of the potential difference to be obtained from such arrangements of solutions, which varies not directly with this ratio but with its logarithm; although for a short range the two methods of variation might agree, and a too limited examination might lead to the inference that the more simply relation was in existence.

Let us therefore take the ratios discovered in these four experiments (Experiment I, .31; Experiment II, .62; Experiment III, 1.21; Experiment IV, 1.51) to exist between the final and initial values of the potential difference, and examine not these figures themselves, but the numbers of which they are the logarithms.

$$\begin{aligned}\text{Experiment I} & \text{— } 0.31 = \log. 2 \\ \text{Experiment II} & \text{— } 0.62 = \log. 4.2 \\ \text{Experiment III} & \text{— } 1.21 = \log. 16.2 \\ \text{Experiment IV} & \text{— } 1.51 = \log. 32.5\end{aligned}$$

Such a collection of figures becomes of immediate interest when it is appreciated that the concentrations of the experimental solutions used in these experiments bear the following ratios to one another :—

Experiment I	Experiment II	Experiment III	Experiment IV
32	16	4	2

it is at once realized that a definite relation has been discovered which unites together completely the very different effects of the solutions of hydrochloric acid used in these experiments.

Final potential difference = initial potential difference $\times \log. \frac{k}{n}$; where
'k' is a constant for all the experiments, and 'n' is the concentration of the solution in gram-molecules per litre—

$$E_w = E_a \log. \frac{k}{n}$$

Needless to say, such a formulated expression of opinion can be readily tested by the use of the data from each of the four experiments, and that in each case the use of these data should lead to the discovery of the same value for 'k.'

EXPERIMENT I

$$\begin{aligned}
 13.46 \times \log. (18.2, k) &= 4.22 \\
 \log. (18.2, k) &= .31 = \log. 2 \\
 \therefore k &= \frac{2}{18.2} = .11
 \end{aligned}$$

EXPERIMENT II

$$\begin{aligned}
 13.99 \log. (36.3, k) &= 8.71 \\
 \log. (36.3, k) &= .62 = \log. 4.16 \\
 \therefore k &= \frac{4.16}{36.3} = .11
 \end{aligned}$$

EXPERIMENT III

$$\begin{aligned}
 15.84 \times \log. (145.2, k) &= 19.27 \\
 \log. (145.2, k) &= 1.21 = \log. 16.2 \\
 \therefore k &= \frac{16.2}{145.2} = .11
 \end{aligned}$$

EXPERIMENT IV

$$\begin{aligned}
 18.48 \log. (290.4, k) &= 28.25 \\
 \log. (290.4, k) &= 1.51 = \log. 32.5 \\
 \therefore k &= \frac{32.5}{290.4} = .11
 \end{aligned}$$

'k' therefore is in reality a constant, and the law which unites the effects of solutions of hydrochloric acid is simple, and is—

$$E_w = E_a \log. \frac{.11}{n}$$

The discovery of such a 'concentration law' has two important results. The first of these is undoubtedly the strong confirmation of the value of the line of reasoning which led to its discovery, namely, that based primarily upon the opinion that the source of E.M.F. of the injury current is due to a difference between the solutions in contact with the electrodes, such as may be described as a solution 'concentration cell.' The second is the strong indication that valuable information is to be obtained from the observed effects of solutions of other electrolytes; if attention is paid to the numbers of which the ratios between the final and the initial potential difference are the logarithms, and not to these ratios themselves. The value of such an indication is seen by a consideration of the experiments given in the preceding table.

Taking the data from this table, let us arrange them in a manner which will test the hypothesis, that the law discovered for hydrochloric acid solutions is not peculiar to it, but is common to all solutions of electrolytes.

$$\frac{E\omega}{E\alpha} = \log. \frac{k}{n}$$

The number, which is the logarithm of the ratio between the final and initial value of the potential difference, is always equal to a constant 'k' divided by the concentration of the experimental solution expressed in gram molecules per litre.

To test such an opinion it is only necessary to obtain the ratio $\frac{E\omega}{E\alpha}$; to find the number of which this value is the logarithm; and to multiply the value thus found by 'n' the concentration.

$$\text{Since } \frac{k}{n} \times n = k.$$

Found in this way, the value 'k' should be constant for each electrolyte.

The following tables of data provide the briefest method of describing the results of this test of the hypothesis.

SOLUTIONS OF AMMONIUM CHLORIDE

Number of Experiment	$\frac{E\omega}{E\alpha}$	$\frac{k}{n}$	n	Therefore k is equal to
Experiment V	·045 = log.	1·10	1	1·10
„ VI	·102 = „	1·26	1	1·26
„ VII	·325 = „	2·11	$\frac{1}{2}$	1·05
„ VIII	·330 = „	2·14	$\frac{1}{2}$	1·07
„ IX	·654 = „	4·51	$\frac{1}{4}$	1·13
„ X	·884 = „	7·66	$\frac{1}{8}$	0·96

$$\therefore \frac{E\omega}{E\alpha} = \log. \frac{1}{n} \text{ (approx.)}$$

SOLUTIONS OF LITHIUM CHLORIDE

Number of Experiment	$\frac{E\omega}{E\alpha}$	$\frac{k}{n}$	n	Therefore k is equal to
Experiment XI	0.268 = log.	1.85	1	1.85
„ XII	0.593 = „	3.92	$\frac{1}{2}$	1.96
„ XIII	0.911 = „	8.15	$\frac{1}{4}$	2.07
„ XIV	1.220 = „	16.60	$\frac{1}{8}$	2.20

$$\therefore \frac{E\omega}{E\alpha} = \log. \frac{2}{n} \text{ (approx.)}$$

SOLUTIONS OF CALCIUM CHLORIDE

Number of Experiment	$\frac{E\omega}{E\alpha}$	$\frac{k}{n}$	n	Therefore k is equal to
Experiment XV	0.354 = log.	2.26	1	2.26
„ XVI	0.648 = „	4.45	$\frac{1}{2}$	2.23
„ XVII	0.876 = „	7.52	$\frac{1}{4}$	1.98

$$\therefore \frac{E\omega}{E\alpha} = \log. \frac{2}{n} \text{ (approx.)}$$

SOLUTIONS OF BARIUM CHLORIDE

Number of Experiment	$\frac{E\omega}{Ea}$	$\frac{k}{n}$	n	Therefore k is equal to
Experiment XVIII ...	0.245 = log.	1.76	1	1.76
„ XIX ...	0.495 = „	3.13	$\frac{1}{2}$	1.57
„ XX ...	0.695 = „	4.96	$\frac{1}{4}$	1.24
„ XXI ...	0.950 = „	8.91	$\frac{1}{8}$	1.14

$$\therefore \frac{E\omega}{Ea} = \log. \frac{1.5}{n} \text{ (approx.)}$$

SOLUTIONS OF SODIUM CHLORIDE

Number of Experiment	$\frac{E\omega}{Ea}$	$\frac{k}{n}$	n	Therefore k is equal to
Experiment XXII ...	0.135 = log.	1.36	1	1.36
„ XXIII ...	0.308 = „	2.03	$\frac{1}{2}$	1.01
„ XXIV ...	0.289 = „	1.95	$\frac{1}{2}$	0.97
„ XXV ...	0.666 = „	4.63	$\frac{1}{4}$	1.16
„ XXVI ...	0.588 = „	3.88	$\frac{1}{4}$	0.97
„ XXVII ...	0.904 = „	8.01	$\frac{1}{8}$	1.00

$$\therefore \frac{E\omega}{Ea} = \log. \frac{1}{n} \text{ (approx.)}$$

SOLUTIONS OF POTASSIUM CHLORIDE

Number of Experiment		$\frac{E\omega}{E\alpha}$	$\frac{k}{n}$	n	Therefore k is equal to
Experiment (a)	...	0.109 = log.	1.28	1	1.28
„ (b)	...	0.407 = „	2.55	$\frac{1}{2}$	1.27
„ (c)	...	0.640 = „	4.40	$\frac{1}{4}$	1.10

$$\therefore \frac{E\omega}{E\alpha} = \log. \frac{1}{n} \text{ (approx.)}$$

It may truly be said that the data from these experiments, arranged in this manner, need little commentary.

The tale which they tell is evidently a simple one, each electrolyte relating its not very different variant.

All these electrolytes, NaCl, KCl, BaCl₂, CaCl₂, LiCl, NH₄ Cl, produce an effect upon the value of the injury current which is mainly dependent upon their concentration; and the effects of different concentrations are in each case united by a simple law which is apparently different for different electrolytes.

The general form of the law is constant throughout the series—

$$\frac{E\omega}{E\alpha} = \log. \frac{k}{n}$$

it would seem, however, that there is a value of 'k' proper to each electrolyte. The determination, therefore, of this value in each case becomes a matter of importance.

The experiments, the records of which are briefly given in the data of the tabulated lists below, were performed to determine in the case of each electrolyte this value 'k'; it being considered temporarily of greater interest to devote time and material to this cause than to the further exploration of the action of other electrolytes. The electrolytes already examined, although all belonging to the same group, chlorides, are sufficiently well known to have materially different influences upon biological phenomena, to make even their agreement in this manner an anomaly difficult of explanation upon any other than purely physical lines. Besides, the same general statement has been found possible as a description of the action of a quite different substance NaOH,* although in this case the form of the concentration law has not yet been determined.

* See preliminary communication, *Proc. Roy. Soc.*, p. 67

Each line in the lists given below represents the data obtained from a separate experiment performed as before, each upon a different sciatic nerve (cat). The solution used was in every case of the concentration of one equivalent gramme molecule per litre ; as will be seen, a large number of separate experiments were performed with such a solution of each of the electrolytes in question.

In the case of each electrolyte, one of the experiments given below has been already quoted. Every such experiment is marked with an asterisk, and numbered as previously.

EXPERIMENTS PERFORMED WITH THE NORMAL SOLUTION OF NaCl

(5.85 grammes per cent.)

(Concentration $n = 1 \therefore \frac{k}{n} = k$)

Number of Experiment	POTENTIAL DIFFERENCE $\times 10^{-3}$ Daniell		Ratio $\frac{E_{\omega}}{E_{\alpha}}$	k
	Initial E_{α}	Final E_{ω}		
* Experiment XXII (1) ...	20.59	2.77	.135 = log.	1.36
„ XXVIII (1) ...	19.40	3.04	.156 = „	1.43
„ XXIX (1) ...	18.22	3.17	.174 = „	1.49
„ XXX (2) ...	17.16	3.04	.178 = „	1.50
„ XXXI (1) ...	16.37	3.17	.193 = „	1.56
„ XXXII (1) ...	15.05	1.72	.114 = „	1.30
„ XXXIII (2) ...	14.92	2.51	.168 = „	1.48
„ XXXIV (1) ...	10.03	0.92	.092 = „	1.24
Average of eight experiments ...	16.47	2.54	.157 = log.	1.42

$$\therefore E_{\omega} = E_{\alpha} \log. 1.42$$

EXPERIMENTS WITH THE NORMAL SOLUTION OF KCl

(7.45 grammes per cent.)

Number of Experiments	POTENTIAL DIFFERENCE x 10-3 Daniell		Ratio $\frac{E_w}{E_a}$	k
	Initial E_w	Final E_a		
Experiment XXXV (1) ...	20.33	2.11	.104 = log.	1.27
„ XXXVI (2) ...	19.27	2.38	.123 = „	1.33
„ XXXVII (1) ...	17.16	1.32	.080 = „	1.21
„ XXXVIII (1) ...	15.84	1.58	.100 = „	1.26
„ XXXIX (2) ...	15.71	2.11	.134 = „	1.36
„ XL (1) ...	15.05	1.72	.114 = „	1.30
„ XLI (2) ...	15.05	1.58	.105 = „	1.27
„ XLII (2) ...	15.18	2.11	.138 = „	1.37
* „ XLIII (1) ...	14.52	1.59	.109 = „	1.28
„ XLIV (2) ...	11.88	1.32	.111 = „	1.29
Average of ten experiments ...	16.08	1.78	.112 = log.	1.30

$$\therefore E_w = E_a \log. 1.30$$

NORMAL SOLUTION OF LiCl

(4.25 grammes per cent)

Number of Experiment	POTENTIAL DIFFERENCE x 10 ⁻³ Daniell		Ratio $\frac{E_{\omega}}{E_{\alpha}}$	k
	Initial E_{α}	Final E_{ω}		
*Experiment XI (1) ...	19.67	5.28	.268 = log.	1.85
„ XLV (1) ...	17.82	6.07	.340 = „	2.19
„ XLVI (2) ...	16.10	4.22	.262 = „	1.83
„ XLVII (1) ...	14.52	3.70	.255 = „	1.80
„ XLVIII (2) ...	12.66	3.83	.302 = „	2.00
Average of five experiments ...	16.15	4.62	.285 = log.	1.93

$$\therefore E_{\omega} = E_{\alpha} \log. 1.93$$

NORMAL SOLUTION OF NH_4Cl .

(5.35 grammes per cent)

Number of Experiment	POTENTIAL DIFFERENCE x 10^{-3} Daniell		Ratio $\frac{E_{\omega}}{E_a}$	k
	Initial E_a	Final E_{ω}		
Experiment XLIX (1) ...	23.23	2.90	.125 = log.	1.33
„ L (2) ...	20.33	1.85	.090 = „	1.23
„ LI (2) ...	18.74	2.38	.127 = „	1.34
„ LII (1) ...	18.61	1.85	.099 = „	1.25
„ LIII (1) ...	17.16	1.58	.092 = „	1.23
„ LIV (2) ...	16.90	1.45	.086 = „	1.22
„ LV (1) ...	16.76	1.45	.086 = „	1.22
„ LVI (2) ...	15.84	1.19	.075 = „	1.19
„ LVII (1) ...	15.05	0.79	.052 = „	1.13
* „ V (1) ...	14.52	0.66	.045 = „	1.10
„ LVIII (2) ...	12.94	0.79	.061 = „	1.15
Average of eleven experiments ...	17.40	1.57	.084 = log.	1.22

$$\therefore E_{\omega} = E_a \log. 1.22$$

NORMAL SOLUTION OF $\frac{1}{2}$ (BaCl_2)

(10.4 grammes per cent.)

12.2 grammes per cent. of the crystalline salt

Number of Experiment				POTENTIAL DIFFERENCE $\times 10^{-3}$ Daniell		Ratio $\frac{E_{\omega}}{E_a}$	k
				Initial E_a	Final E_{ω}		
Experiment LIX	(1)	...		19.01	3.96	.208 = log.	1.61
„ LX	(1)	...		17.95	4.49	.250 = „	1.78
* „ XVIII	(1)	...		17.69	4.36	.245 = „	1.76
„ LXI	(1)	...		16.90	3.17	.187 = „	1.54
„ LXII	(2)	...		16.76	4.49	.267 = „	1.85
„ LXIII	(2)	...		16.10	3.96	.246 = „	1.76
„ LXIV	(2)	...		13.46	3.56	.268 = „	1.85
„ LXV	(1)	...		12.67	2.64	.208 = „	1.62
„ LXVI	(2)	...		11.88	3.17	.266 = „	1.84
Average of nine experiments				15.62	3.75	.238 = log.	1.73

$$\therefore E_{\omega} = E_a \log. 17.3$$

NORMAL SOLUTION OF $\frac{1}{2}$ (MgCl₂)

(4.75 grammes per cent.)

10.15 grammes per cent. of the crystalline salt

Number of Experiment	POTENTIAL DIFFERENCE x 10-3 Daniell		Ratio $\frac{E_{\omega}}{E_{\alpha}}$	k
	Initial E_{α}	Final E_{ω}		
Experiment LXXVII (1) ...	22.18	6.60	.297 = log.	1.97
„ LXXVIII (2) ...	20.20	6.07	.300 = „	2.00
„ LXXIX (1) ...	19.80	5.28	.266 = „	1.84
„ LXX (2) ...	18.22	5.41	.296 = „	1.98
„ LXXI (1) ...	17.69	4.49	.246 = „	1.76
„ LXXII (2) ...	17.42	5.02	.288 = „	1.94
„ LXXIII (1) ...	16.50	5.02	.304 = „	2.01
„ LXXIV (1) ...	15.31	4.75	.310 = „	2.04
„ LXXV (2) ...	14.52	4.22	.290 = „	1.95
„ LXXVI (1) ...	14.12	2.64	.187 = „	1.54
„ LXXVII (2) ...	12.67	3.04	.240 = „	1.74
Average of eleven experiments ...	17.15	4.78	.275 = log.	1.89

$$\therefore E_{\omega} = E_{\alpha} \log. 1.89$$

NORMAL SOLUTION OF $\frac{1}{2}$ (CaCl₂)

(5.55 grammes per cent. of anhydrous salt)

Number of Experiment	POTENTIAL DIFFERENCE x 10-3 Daniell		Ratio $\frac{E_{\omega}}{E_a}$	k
	Initial E _a	Final E _ω		
* Experiment XV (1) ...	19.44	6.88	.354 = log.	2.26
„ LXXVIII (1) ...	18.48	6.34	.343 = „	2.20
„ LXXIX (2) ...	15.05	5.28	.351 = „	2.24
„ XC (2) ...	13.73	5.28	.384 = „	2.42
„ XCI (1) ...	12.94	3.96	.306 = „	2.02
Average of five experiments ...	15.93	5.55	.348 = log.	2.23

$$\therefore E_{\omega} = E_a \log. 2.23$$

SUMMARY OF PRECEDING RESULTS

The average result of an immersion of five minutes duration in a normal (1 gramme equivalent molecule per litre) solution of	Is to give a new value to the 'injury current,' which can be expressed as being equal to the initial value multiplied by the following factor	This factor can be expressed also in the form given below
NaCl	.151	log. 1.42
KCl	.112	„ 1.30
LiCl	.285	„ 1.93
NH ₄ Cl	.084	„ 1.22
$\frac{1}{2}$ (BaCl ₂)	.238	„ 1.73
$\frac{1}{2}$ (MgCl ₂)	.275	„ 1.89
$\frac{1}{2}$ (CaCl ₂)	.348	„ 2.23

Assuming it to have been proved, that in the case of each electrolyte there is a concentration law' uniting the effects of solutions of this electrolyte in every possible concentration upon the value of the injury current, and that this law is always of the general form—

$$E_{\omega} = E_a \log. \frac{k}{n},$$

where 'n' is the concentration : then in the case of each electrolyte given above we have a different value for the constant 'k.'

Since, in the preceding experiments, $n = 1 \therefore \log. \frac{k}{n} = \log. k$.

NaCl	...	k = 1.42
KCl	...	k = 1.30
LiCl	...	k = 1.93
NH ₄ Cl	...	k = 1.22
$\frac{1}{2}$ (BaCl ₂)	...	k = 1.73
$\frac{1}{2}$ (MgCl ₂)	...	k = 1.89
$\frac{1}{2}$ (CaCl ₂)	...	k = 2.32

The 'concentration law' is evidently not greatly different in the case of these different electrolytes, the value of the constant 'k' varying with each electrolyte to a not very remarkable extent. It will be seen that an allowance made for the influence of an important factor, not yet considered, brings even these differences approximately to naught, and the action of the different electrolytes within the bounds of a law common to them all.

If the action of all these solutions is a purely physical one and dependent upon their electrical properties alone, then we have hitherto been assessing the concentration of the different solutions at a mistaken value. The salt which is in solution as such, and is not dissociated by the fact of solution, is of no account from the purely electrical point of view, consisting, as it does, of neutral molecules. We are alone concerned with the other moiety of the salt, which has been dissociated by the fact of solution into positively and negatively charged particles, hydrolysed into 'ions.' Such a reflection discovers for us a method of regarding the so-called 'equivalent' solutions of electrolytes (used in the experiments of the preceding section) in which they are seen as no longer equivalent, and which points to the necessity of still further checking the results obtained by their use. The concentrations of the solutions used have yet to be brought to a common standard in terms of the dissociated ions contained in them, before the results are made strictly comparable.

'The degree of dissociation of a substance in a solution is equal to the ratio of the equivalent conductivity of that solution to its equivalent conductivity at infinite dilution.'¹ Fortunately, tabulated lists of such equivalent conductivities obtained by the experimental work of many investigators, have been prepared by FITZPATRICK,² to which we may conveniently refer (the same lists are also found as an appendix to WHETHAMS' *Solution and Electrolysis*). From these lists the following data have been collected and used in the determination of the dissociation constants :—

Electrolyte	Molecular conductivity of the 'equivalent' solution	Molecular conductivity at infinite dilution	Dissociation constant
NaCl	695	1024	·68
KCl	919	1216	·75
NH ₄ Cl	907	1215	·75
$\frac{1}{2}$ (BaCl ₂)	658	1144	·58
$\frac{1}{2}$ (MgCl ₂)	631*	1070*	·59
$\frac{1}{2}$ (CaCl ₂)	633	1043	·60
LiCl	591	965	·61

The normal solution of NaCl therefore used in the experiments of the preceding section, did not, as we have formerly represented it, contain one equivalent gramme molecule per litre of important material, but only ·68 of this.

The value of 'k' obtained from the result, $\frac{k}{n} = 1\cdot42$, is not $k = 1\cdot42$, since 'n' is not equal to 1, but to ·68,

$$\begin{aligned}\therefore k &= 1\cdot42 \times \cdot68 \\ &= \cdot97\end{aligned}$$

The values of 'k' obtained for each of the other electrolytes has similarly to be corrected by the use of the dissociation constants given above, the results of this correction are given on following page.

1. Le Blanc, *Electro-chemistry*, 87, transl.
2. *British Association Reports*, 1893.

Electrolyte	Dissociation constant	Corrected value of k
NaCl	·68	$1.42 \times .68 = 0.97$
KCl	·75	$1.30 \times .75 = 0.95$
NH ₄ Cl	·75	$1.22 \times .75 = 0.92$
$\frac{1}{2}$ (BaCl ₂)	·58	$1.73 \times .58 = 1.00$
$\frac{1}{2}$ (MgCl ₂)	·59	$1.89 \times .59 = 1.11$
$\frac{1}{2}$ (CaCl ₂)	·60	$2.23 \times .60 = 1.34$
LiCl	·61	$1.93 \times .61 = 1.17$

In this list there is little need to call attention to the extreme similarity in action upon the injury current of the solutions of these electrolytes. Their action is not only similar in a general sense, but in an exact quantitative sense; in each case that action following a general law

$$E_{\omega} = E_a \log. \frac{1}{n}$$

It may be said that this is only approximately general, the approximation is, however, sufficiently close—

Electrolyte	Concentration Law
NaCl	$\frac{E_a}{E_{\omega}} = \log. \frac{.97}{n}$
KCl	„ = „ $\frac{.95}{n}$
NH ₄ Cl	„ = „ $\frac{.92}{n}$
$\frac{1}{2}$ (BaCl ₂)	„ = „ $\frac{1.00}{n}$
$\frac{1}{2}$ (MgCl ₂)	„ = „ $\frac{1.11}{n}$
$\frac{1}{2}$ (CaCl ₂)	„ = „ $\frac{1.34}{n}$
LiCl	„ = „ $\frac{1.17}{n}$

The approximation is sufficiently close to point to a very definite moral ; namely, that the actions of all these solutions are in very definite agreement, and depend upon a property common to them all.

The property can also be assigned definitely as attributable to the dissociated moiety of these electrolytes, to the electrically charged ions contained in these solutions ; since it is obviously dependent upon the number of ions present.

Further, there remains the remarkable fact, not yet commented upon, that the form of the law determines one concentration which should reduce the potential difference to zero : and that this particular concentration, since $\log. 1 = 0$, is $n = 1$.

A solution of concentration $n = 1$, one equivalent gramme molecule per litre of dissociated electrolyte should cause the injury current to vanish. Such a necessity logically carries us to an extraordinary conclusion, when attention is paid to the main contention which we have attempted to establish : namely, that the current of injury is due to the inequality between the 'external' and 'internal' solution of the nerve. For equality of these two solutions is the condition essential to the elimination of the injury current ; and it must be admitted that, with the figure just given, equality is reached at an extraordinary concentration of the 'external solution.'

Such a conclusion necessitates a very rigid examination of the 'concentration law,' and this seems best performed by an exhaustive examination of the action of one of those electrolytes over a wide range of concentration.

There does not seem to be any great advantage obtainable from the choice of a special electrolyte, the action of all so far being similar. For this examination KCl has been chosen thus at random.

SOLUTIONS OF POTASSIUM CHLORIDE

SPECIAL EXAMINATION OF THE ACTION OF SOLUTIONS OF POTASSIUM CHLORIDE

The action of solutions of the concentration of one gramme equivalent molecule per litre (7.45 grammes per cent.) has already been completely examined (see p. 316), the average result being

$$E_{\omega} = E_a \log 1.42.$$

This relation differently expressed being

$$E_{\omega} = E_a \log \frac{.97}{n}$$

where 'n' represents the concentration in gramme molecules per litre of the dissociated portion of electrolyte (.68).

The following results, with this, cover an examination of a sufficiently wide range of concentrations, from 7.45 grammes per cent. to 0.18 grammes per cent.

The experimental method followed is precisely that previously detailed, and each observation recorded is from an individual sciatic nerve (cat) used for this observation (injury current before and after immersion in the given solution) and for no other.

KCL (3.72 GRAMMES PER CENT.)

($\frac{1}{2}$ gramme equivalent molecule per litre.)

Number of Experiment				POTENTIAL DIFFERENCE x 10-3 Daniell		Ratio $\frac{E_{\omega}}{E_a}$	$\frac{k}{n}$
				Before Immersion E_a	After Immersion E_{ω}		
Experiment	XCII	(1)	...	24.29	9.90	.408 = log.	2.56
,,	XCIII	(2)	...	17.29	8.45	.489 = „	3.08
,,	XCIV	(1)	...	17.16	5.68	.331 = „	2.14
,,	XCV	(2)	...	14.78	5.02	.339 = „	2.19
,,	XCVI	(1)	...	14.52	6.60	.454 = „	2.85
,,	XCVII	(2)	...	13.60	6.46	.475 = „	2.99
,,	XCVIII	(1)	...	18.88	9.00	.479 = „	3.01
,,	XCIX	(2)	...	16.37	7.79	.475 = „	2.99
,,	C	(1)	...	18.22	8.18	.449 = „	2.81
,,	CI	(2)	...	19.27	9.37	.486 = „	3.06
Average of ten experiments ...				17.44	7.65	.438 = log.	2.74
Average of five experiments marked (1)				18.61	7.87	.423 = „	2.65
Average of five experiments marked (2)				16.26	7.48	.460 = „	2.89

KCL (1.86 GRAMMES PER CENT.)

($\frac{1}{4}$ gramme equivalent molecule per litre)

Number of Experiment				POTENTIAL DIFFERENCE $\times 10^{-3}$ Daniell		Ratio $\frac{E_{\omega}}{E_a}$	$\frac{k}{n}$
				Before Immersion E_a	After Immersion E_{ω}		
Experiment CII	(1)	...		22.18	11.15	.503 = log.	3.19
„ CIII	(2)	...		21.38	10.96	.512 = „	3.24
„ CIV	(1)	...		17.42	9.77	.561 = „	3.64
„ CV	(1)	...		22.18	12.94	.583 = „	3.83
„ CVI	(2)	...		16.90	12.14	.718 = „	5.23
„ CVII	(1)	...		19.01	9.50	.500 = „	3.16
„ CVIII	(2)	...		16.90	10.82	.640 = „	4.37
„ CIX	(1)	...		14.78	9.50	.643 = „	4.40
„ CX	(2)	...		15.05	10.82	.718 = „	5.23
„ CXI	(1)	...		13.46	9.24	.686 = „	4.86
„ CXII	(2)	...		13.20	8.45	.640 = „	4.37
Average of eleven experiments ...				17.40	10.48	.510 = log.	4.14
Average of six experiments marked (1)				18.17	10.35	.569 = „	3.71
Average of five experiments marked (2)				16.68	10.64	.638 = „	4.35

KCL (1.49 GRAMMES PER CENT.)

($\frac{1}{5}$ gramme equivalent molecule per litre.)

Number of Experiment	POTENTIAL DIFFERENCE $\times 10^{-3}$ Daniell		Ratio $\frac{E_{\omega}}{E_{\alpha}}$	$\frac{k}{n}$
	Before Immersion E_{α}	After Immersion E_{ω}		
Experiment CXIII (1) ...	15.05	10.56	.701 = log.	5.03
„ CXIV (2) ...	13.20	9.11	.690 = „	4.90
„ CXV (1) ...	22.70	15.58	.686 = „	4.86
„ CXVI (2) ...	17.42	12.14	.697 = „	4.98
„ CXVII (1) ...	19.54	14.78	.756 = „	5.71
„ CXVIII (2) ...	18.48	13.20	.714 = „	5.18
„ CXIX (1) ...	18.48	10.56	.571 = „	3.73
„ CXX (2) ...	20.06	12.14	.605 = „	4.03
Average of eight experiments ...	18.11	12.26	.677 = log.	4.76
Average of four experiments marked (1)	18.94	12.87	.679 = „	4.78
Average of four experiments marked (2)	17.29	11.65	.674 = „	4.73

KCL (·745 GRAMMES PER CENT.)

(1₁₀ gramme equivalent molecule per litre.)

Number of Experiment			POTENTIAL DIFFERENCE x 10 ⁻³ Daniell		Ratio $\frac{E_{\omega}}{E_a}$	$\frac{k}{n}$
			Before Immersion E_a	After Immersion E_{ω}		
Experiment CXXI	(1)	...	18·74	20·20	1·078 = log.	11·97
„ CXXII	(2)	...	16·10	16·63	1·033 = „	10·79
„ CXXIII	(1)	...	21·38	19·93	0·932 = „	8·55
„ CXXIV	(2)	...	17·42	18·22	1·046 = „	11·12
„ CXXV	(1)	...	15·58	16·24	1·042 = „	11·02
„ CXXVI	(2)	...	13·20	17·95	1·359 = „	22·86
„ CXXVII	(1)	...	20·20	17·42	0·862 = „	7·28
„ CXXVIII	(2)	...	17·42	18·74	1·076 = „	11·92
„ CXXIX	(1)	...	18·22	16·63	0·913 = „	8·19
„ CXXX	(2)	...	15·05	17·16	1·140 = „	13·81
„ CXXXI	(1)	...	26·66	22·18	0·832 = „	6·79
„ CXXXII	(2)	...	21·91	25·08	1·145 = „	13·97
„ CXXXIII	(1)	...	15·84	17·95	1·133 = „	13·59
„ CXXXIV	(2)	...	15·05	17·69	1·175 = „	14·97
„ CXXXV	(1)	...	15·05	17·69	1·175 = „	14·97
„ CXXXVI	(2)	...	17·69	16·63	0·940 = „	8·71
„ CXXXVII	(1)	...	16·90	14·78	0·874 = „	7·48
„ CXXXVIII	(2)	...	15·84	14·78	0·933 = „	8·57
„ CXXXIX	(1)	...	15·31	14·52	0·948 = „	8·87
„ CXL	(2)	...	15·84	16·37	1·033 = „	10·79
Average of twenty experiments	...		17·47	17·84	1·021 = log.	10·5
Average of ten experiments marked (1)			18·39	17·75	0·965 = „	9·23
Average of ten experiments marked (2)			16·55	17·93	1·083 = „	12·11

So far as the examination of the action of solutions of potassium chloride has been carried, the 'concentration law,' as determined in the preceding sections, holds good, and serves to combine the results obtained. The range of concentrations examined has been, it will be acknowledged, fairly extensive, from 7.45 grammes per cent. to 0.745 grammes per cent. *The whole range of concentrations used, with the exception of the last example, however, is above that of the isotonic solution.*

In the following table the average results of these experiments with different concentrations of potassium chloride solution are arranged. The comparison, so facilitated, will be found to amply vindicate the truth of the 'concentration law.'

AVERAGE RESULTS TABULATED

CONCENTRATION OF THE SOLUTION		The dissociation constant at this concentration	The Ratio between the Final and Initial Potential Differences	The general 'Concentration Law' as defined by the data from each special case (value of 'n' corrected by the use of the dissociation constant)
In grammes per cent.	In gramme equivalent molecules per litre			
(a) 7.45	1	.68	log. 1.30	$\frac{E\omega}{E\alpha} = \log. \frac{.97}{n}$
(b) 3.72	$\frac{1}{2}$.78	" 2.74	" = " $\frac{1.07}{n}$
(c) 1.86	$\frac{1}{4}$.85	" 4.14	" = " $\frac{.85}{n}$
(d) 1.86	$\frac{1}{5}$.85	" 4.76	" = " $\frac{.82}{n}$
(e) 0.745	$\frac{1}{10}$.86	" 10.50	" = " $\frac{.91}{n}$

The general agreement is shown by the last column of the preceding table. It will be seen to be in marked contrast to the results obtained from the action of solutions much below the 'isotonic solution' in concentration, as shewn by the experiments collected in the following tables. A glance at the .745 KCl table will show how great the individual exceptions are which are found at this concentration. The truth of the law is here only indicated by an appeal to the average result obtained: which is, however, conclusive. Such an appeal to an average is only of value when no liberty is retained to eliminate undesirable cases from the list, and this method has been strictly followed in the .745 table. It has also been strictly adhered to in tabulating the results obtained with solutions still more dilute than the isotonic, the results of which are given in the two following tables.

KCL (0.372 GRAMMES PER CENT.)

($\frac{1}{25}$ gramme equivalent molecule per litre)

Number of Experiment			E_a $\times 10^{-3}$ Daniell	E_w $\times 10^{-3}$ Daniell	$\frac{E_w}{E_a}$
Experiment CXL	(1)	...	15.84	17.42	1.09
„ CXLI	(2)	...	13.46	14.78	1.09
„ CXLII	(1)	...	23.76	24.42	1.03
„ CXLIII	(2)	...	24.02	24.02	1.00
„ CXLIV	(1)	...	17.42	19.01	1.09
„ CXLV	(2)	...	15.51	16.37	1.06
„ CXLVI	(1)	...	18.22	19.14	1.05
„ CXLVII	(2)	...	13.86	15.05	1.09
„ CXLVIII	(1)	...	19.01	20.46	1.08
„ CXLIX	(2)	...	16.10	17.42	1.08
„ CL	(1)	...	21.52	19.54	0.91
„ CLI	(2)	...	16.37	18.48	1.13
„ CLII	(1)	...	21.91	21.91	1.00
„ CLIII	(2)	...	19.54	21.65	1.11
„ CLIV	(1)	...	17.42	19.54	1.12
„ CLV	(2)	...	17.42	19.27	1.11
„ CLVI	(1)	...	17.42	18.74	1.08
„ CLVII	(2)	...	16.63	19.80	1.19
„ CLVIII	(1)	...	17.69	15.84	0.89
„ CLIX	(2)	...	16.63	16.63	1.00
Average of twenty experiments			17.99	18.98	1.055 = log. 11.4
Average of ten experiments, marked (1)			19.02	19.60	1.030 = „ 10.8
Average of ten experiments, marked (2)			17.00	18.35	1.079 = „ 12.0

KCL (0.186 GRAMMES PER CENT.)

 $(\frac{1}{40}$ gramme equivalent molecule per litre).

Number of Experiment			E_a $\times 10^{-3}$ Daniell	$\times 10^{-3}$ Daniell E_w	$\frac{E_w}{E_a}$
Experiment CLX	(1)	...	19.80	23.50	1.19
„ CLXI	(2)	...	19.27	22.44	1.16
„ CLXII	(1)	...	19.27	21.12	1.10
„ CLXIII	(2)	...	17.16	21.65	1.26
„ CLXIV	(1)	...	22.18	23.76	1.07
„ CLXV	(2)	...	17.16	22.18	1.29
„ CLXVI	(1)	...	20.06	20.59	1.02
„ CLXVII	(2)	...	17.95	20.06	1.12
„ CLXVIII	(1)	...	17.95	18.74	1.04
„ CLXIX	(2)	...	17.69	20.33	1.15
„ CLXX	(1)	...	17.95	19.54	1.09
„ CLXXI	(2)	...	17.16	19.01	1.11
„ CLXXII	(1)	...	21.65	25.08	1.16
„ CLXXIII	(2)	...	19.01	21.91	1.15
„ CLXXIV	(1)	...	19.54	21.12	1.08
„ CLXXV	(2)	...	18.22	23.76	1.30
„ CLXXVI	(1)	...	18.22	22.97	1.26
„ CLXXVII	(2)	...	14.52	24.02	1.65
„ CLXXVIII	(1)	...	16.90	21.91	1.29
„ CLXXIX	(2)	...	14.52	20.33	1.40
Average of twenty experiments ...			18.11	21.70	1.198 = log. 16
Average of ten experiments marked (1)...			19.35	21.80	1.129 = „ 13.5
Average of ten experiments marked (2)...			17.26	21.56	1.249 = „ 17.8

An examination of the experimental observations tabulated in the two last tables establishes some confidence in the average uniformity of action of the two solutions upon the injury current of nerve. Either solution may be truly said to produce an effect characteristic to itself and different from that produced by solutions of different concentration.

The results obtained are not, however, as great as was anticipated from the point of view justified by the examination of solutions of greater concentration, namely, that the results of all solutions of KCl could be summed up in a universally applicable 'concentration law,'

$$\frac{E_{\omega}}{E_a} = \log \frac{1}{n} \text{ (approx.)}$$

Thus in the case of solutions of .372 grammes per cent. KCl, the concentration in gramme molecules per litre, n , is equal to $\frac{1}{20}$. The dissociation constant at this concentration is .9. The concentration of the dissociated moiety of the KCl is nearly that of the total KCl present,

$$\begin{aligned} \frac{.9}{20} \\ \therefore \frac{E_{\omega}}{E_a} &= \log \frac{1}{\frac{.9}{20}} \\ &= \log. 22.22 \\ E_{\omega} &= 1.345 \times E_a \end{aligned}$$

But the actual result of the experiments was not this; the final value was smaller than such anticipation suggested, and was

$$\begin{aligned} E_{\omega} &= 1.055 \times E_a \\ \text{or } E_{\omega} &= E_a \log. 11.4 \end{aligned}$$

Similarly, in the case of .186 grammes per cent. solutions of KCl ($\frac{1}{40}$ gram. mol.), the anticipation of the general law is not fulfilled; is, indeed, further from attainment than in the last case.

Thus the dissociation constant at this concentration is .93 (approx.)

$$\begin{aligned} n &= \frac{.93}{40} \\ \therefore E_{\omega} &= E_a \log. \frac{1}{\frac{.93}{40}} \\ &= E_a \log. 43 \\ &= E_a \times 1.63 \end{aligned}$$

Whereas the result actually obtained was—

$$\begin{aligned} E_{\omega} &= E_a \times 1.20 \\ E_{\omega} &= E_a \log. 16. \end{aligned}$$

Nor is this the most severe way in which the difference between anticipation and reality could be described. An examination of the first of these two tables will show that in this series of experiments the anticipated result was not even once approached; that in the second table the anticipated result was only once obtained, and then in an experiment upon a 'second nerve' (Experiment CLXXVII). The universality of the general 'concentration law' has, therefore, broken down, while still the general statements made as to the graduated effects of solutions of different concentration are unaffected, as will be seen from the collection of the results in the following table:—

SOLUTIONS OF POTASSIUM CHLORIDE

Concentration in grammes per cent.	Number of Sciatic Nerves examined	Average initial Potential Difference	Average final Potential Difference	The Final Value expressed in terms of the Initial Value as unity
7.45	10	.0160 Daniell	.0018 Daniell	0.11
3.72	10	.0174 „	.0076 „	0.44
1.86	11	.0174 „	.0105 „	0.60
1.49	8	.0181 „	.0123 „	0.68
0.75	20	.0175 „	.0179 „	1.02
0.37	20	.0180 „	.0190 „	1.06
0.19	20	.0181 „	.0217 „	1.20

It has been pointed out (p. 325) that the 'concentration law' has an interest of much greater magnitude than that given to it by the fact, that it successfully describes the effects of solutions within a wide range of concentrations.

It has a value which is given to it by its own form. The form is, in the first place, a confirmation of the position which has been maintained in this paper as regards the method of production of the injury current. In the second place, the actual numerical values of the expressions contained in this law have a very great interest; for no matter what the explanation of the quantitative relation discovered between the results of immersion in solutions of varied concentration, whether it is the one here taken or some other, the expression $Ea = E\omega \log. \frac{I}{n}$ gives rise intrinsically to a most important question.

This expression (since $\log. 1 = 0$) predicts a value for the solution, an immersion in which should reduce the value of the injury current to zero; and the prediction is a very

remarkable one. For the value of the predicted concentration is one gramme equivalent molecule (dissociated) per litre, and is, therefore, very great; being that of a solution capable of exerting an osmotic pressure ten times greater than that of ordinary 'normal saline' solution.

If, therefore, the theory here maintained is the true one, if the injury current is due to the contrast between internal and external solution, if also the electrolytes of this internal solution are not very different from the chlorides examined; then the concentration law, as found, points to the existence of a solution of this extraordinary concentration within the axis cylinders of the nerve fibres.

If on the other hand, the theory now maintained and supported by so much circumstantial evidence is false; then, if still the 'concentration law' holds good, *an explanation has to be found for this relationship explanatory of another indication which it holds forth, namely, that a solution of greater concentration still must reverse the direction of the injury current.*

For take the expression $E_{\omega} = E_a \log \frac{k}{n}$; in this expression when 'n' is greater than 'k,' then $\log \frac{k}{n}$ becomes negative, and with it necessarily also the value of E_{ω} , the final potential difference after immersion.

In illustration of this necessity the following experiments with solutions of NaOH are given. The data given are the results of experiments upon sciatic nerves (cats) exactly similar to the preceding ones, and like them shewing the effect of immersions of five minutes duration.

SOLUTIONS OF NaOH

Number of Experiment		Concentration of the Solution in grammes per cent.	Initial Potential Difference $\times 10^{-3}$ Daniell	Final Potential Difference $\times 10^{-3}$ Daniell	Final Value expressed in terms of the Initial Value as unity
*	(I) ...	0.025	23.0	+ 23.9	+ 1.04
Experiment CLXXX	(2) ...	0.063	13.2	+ 20.1	+ 1.60
„ CLXXXI	(I) ...	0.125	13.2	+ 15.0	+ 1.14
*	(I) ...	0.250	20.1	+ 9.0	+ 0.45
„ CLXXXII	(I) ...	0.500	20.9	+ 1.9	+ 0.09
„ CLXXXIII	(2) ...	1.000	20.3	— 1.3	— 0.06
„ CLXXXIV	(I) ...	1.000	13.3	— 2.3	— 0.16

* Experiments previously quoted on pages 298, 302

I have shewn previously* that the results of more prolonged immersions in solutions of NaOH follow the same law (general statement) as that followed by the results of such electrolytes as NaCl, KCl, HCl. The experiments given above are the only ones which I have performed with immersions of short duration, and are given without exception. If attention is paid to the ratios in the last column, then, the first experiment excepted, the experimental results form an excellent series of values declining to a complete reversal. If attention is paid to the final differences of potential alone then there is no exception, and such a course is justified by the data and observations of page 300. Exception or not, the fact of the reversal is definite, and is confirmed by repetition.

The form, therefore, of the 'concentration law' is of the utmost importance. Is it to be considered as unfavourably affected by the failure to bring within its limits the results of experiments with solutions of .37 and .19 grammes per cent. potassium chloride? Without hesitation one answers that it is not, and this answer is based upon evidence already exhibited in this paper. *In the first place*, consider the curves of fig. A, page 292, and the experiments from which they were drawn, which shew the anomalous effect of solutions less concentrated than the 'isotonic' solution. The same anomaly has undoubtedly here presented itself in this attempt at quantitative comparison.

The evidence of those earlier experiments is, however, decisive in its indication that the anomalous variation is secondary to a primary normal variation capable of anticipation upon the lines there indicated and now more definitely formulated in the 'concentration law.' The considerations there advanced in explanation of this secondary anomaly may be summarized in the statement—*that the decline of the injury current is more rapid in solutions below the 'isotonic' solution in concentration, because the diffusion of electrolytes out of the axis cylinders of the nerve is more complete.* The decline in this case is, as was then shewn, a real one, and is in contrast to the apparent decline which is experienced in concentrated solutions.

It has been previously asserted (page 303) that the final value after immersion should be compared, not to the initial value, but to the value as affected by the normal decline, and that there is no means of doing this. The necessity for such a correction is greater still when it is acknowledged that the experimental modifications used are inevitably themselves productive of variations in this rate of decline.

The error cannot therefore be allowed for, and necessarily limits the possible range of experiment: for its mode of action is capable of prediction, but not its quantitative value. That the error has been met with in these experiments with dilute solutions is a fact to be recognized, and regretted, but in no way can it be allowed to detract from the value of the remaining experiments.

* Preliminary communication, *Proceedings Royal Society*, vol. 67, p. 322.

In the second place, the results obtained by the immersion of nerves in hydrochloric acid (page 309) strongly reinforce such a line of argument. In that instance the widest range of effects, from extreme increase to extreme decrease of the injury current, was obtained by the use of a set of solutions all much more dilute than the 'isotonic' solution. All these solutions practically formed 'vacua' into which the electrolytes of the nerve diffused with great rapidity, the limit to the extent being determined by the time of immersion and being practically the same in each case.

In this case the effect of the error is not obvious, because it is always maximal and the same in the results of every solution used. It is not obvious until the actual quantities in the 'concentration law' for hydrochloric acid solution is examined.

$$\frac{E\omega}{E\alpha} = \log. \frac{1}{n}$$

The value of k in this example is minimal. One of the reasons which contributes to this diminution is the factor capable of definite anticipation, namely, the dilution of the 'internal solution' of the nerve by rapid diffusion processes.

The form of the 'concentration law' is always the same; conclusions deducible from its form, such as the possibility of reversing the direction of the injury current are, therefore, inevitable. The actual value of the constant ' k ' which it contains

$$\frac{E\omega}{E\alpha} = \log. \frac{k}{n}$$

is the same for a very great range of concentrations, and is only varied by conditions which change permanently the value of the source of the injury current. *The meaning of this value ' k ' is fixed by the form of the law, when $k = n$ the injury current vanishes; ' k ' therefore is the concentration of the solution, an immersion in which will reduce the value of the injury source to zero, and necessarily is different for different conditions of the nerve.*

The particular value of ' k ,' which is of the greatest interest, is the value which is true for the effects of immersion in solutions which produce the least permanent effect upon the conditions of the nerve, that is for the value obtained by experiments with solutions as near the 'isotonic' solution in concentration as possible. The range of concentration, which is suggested as being most worthy of accurate experiment, is from one-fifth to one-tenth gramme molecule per litre of the chloride solutions which have been examined.

From this point of view the following series of experiments with one-eighth gramme molecule KCl seem worthy of especial attention.

In order that the real quantitative value of these results may be of permanent interest, they also are given as they were taken in succession, and are given, without exception, from a series of experiments performed one after another in the course of three days. It will be seen that the initial values are greater than usual, a fact which

is probably explainable in terms of the temperature of the air in which the observations were made, 23.5°C (July 16, 1901). The solutions were, of course, kept at the standard temperature of 18°C , but the actual measurements were necessarily affected by the temperature of the electrodes and of the moist chamber which were at the temperature of the room.

This average initial value, greater than usual, has induced me to withhold these experiments from their proper place amidst those previously given; where, had it been possible, I would have preferred to have produced tables in which the experiments were in each case so many and so distributed over different periods of the year (different room temperatures) that the average initial value observed should have been in each case the same. For then the average final values can be expressed in terms of the general average initial value.

KCL (.93 GRAMMES PER CENT.)

($\frac{1}{8}$ gramme equivalent molecule per litre)

Number of Experiment	E_a	E_{ω}	$\frac{E_{\omega}}{E_a}$	$\frac{k}{n}$
Experiment CLXXXV (I) ...	22.44	20.59	0.917 = log.	8.27
„ CLXXXVI (I) ...	21.91	21.12	0.964 = „	9.20
„ CLXXXVII (I) ...	21.91	20.59	0.940 = „	8.71
„ CLXXXVIII (I) ...	21.91	19.54	0.892 = „	7.80
„ CLXXXIX (I) ...	22.70	23.22	1.023 = „	10.55
„ CXC (I) ...	22.57	20.33	0.900 = „	8.00
„ CXCI (I) ...	26.66	24.29	0.911 = „	8.15
Average of seven experiments all upon nerves marked (I) ...	22.87	21.38	0.935 = log.	8.61

$$E_{\omega} = E_a \log. 8.61.$$

From this case then

$$\frac{k}{n} = 8.61$$

and, since $n = \frac{1}{8} \times .85$, the dissociation factor at this concentration being .85, therefore

$$k = \frac{8.61}{8} \times .85 \\ = .91$$

and the 'concentration law,' judged from this instance, is

$$E_{\omega} = E_a \log. \frac{.91}{n}$$

The concentration of the solution, therefore, which is indicated by this result as likely to annul the injury current, is '91 gramme molecule (dissociated) per litre of potassium chloride, that is a solution of approximately 10 grammes per cent. of KCl.

Contrast such a result, which is in complete agreement with those obtained from the majority of the examples previously given, with the conclusion to be derived from an examination of the 'concentration law' for a range of extremely dilute solutions, '2 grammes per cent. KCl, etc. There, judging from the example given on page 332, the 'concentration law' is

$$\begin{aligned} E_{\omega} &= E_{\alpha} \log. 16 \\ n &= \frac{1}{16} \times .94 \\ \therefore E_{\omega} &= E_{\alpha} \log. \frac{.37}{n} \end{aligned}$$

The concentration of the annulling solution here indicated is '37 gramme molecules (dissociated) per litre of potassium chloride, that is a solution of approximately 3.5 grammes per cent of KCl.

Why should a nerve, which has been immersed for five minutes in an almost 'isotonic' solution, be so repeatedly drawn into prophesying the annihilation of this phenomenon of the injury current as the result of an immersion of similar duration in 10 grammes per cent. KCl, and a reversal of the phenomenon as the result of immersion in solutions stronger than this: whereas a nerve which has been immersed for five minutes in a very dilute solution, far below the concentration of the isotonic solution, prophesies a similar doom for itself when less stringent measures are used against it, namely, when it is immersed in 4 grammes per cent. KCl?

The answer, suggested by many facts exhibited in this research, and also by its absolute inherent probability, is that the reason is to be found in the removal of electrolytes from the nerve. A removal which has permanently diminished the value of the structures giving rise to the injury current, and 'weakened the nerve' by diluting the internal solutions of the axis cylinders.

THE CONDUCTIVITY OF THE INTERNAL SOLUTION

If, as has been assumed in the previous section, the immersion of a nerve in solutions approximately isotonic with 'normal saline,' and in solutions slightly more concentrated than this, does not affect to any appreciable extent the pre-existing concentration of the internal solutions; it should be possible by measurements of the conductivity before and after the immersion to calculate the amount of this which is due to the 'internal solution.'

The data from the following two experiments may serve this purpose in a preliminary fashion, sufficing until they can, as is intended, be repeated with solutions of other concentrations.

The first experiment given below, A, serves to shew that a nerve which has been immersed in a .745 grammes per cent. solution of potassium chloride is practically unaltered, as far as its conductivity is concerned, by the immersion; just as in the last section it was shewn that it is unaltered as far as the difference of potential between its longitudinal surface and cross section is concerned.

The experiment is produced mainly so that it may be seen that the basis of the calculation made upon data of the subsequent experiment, B, is not very far from accuracy. This basis being the assumption that the conductivity of the external solution pre-existent upon the nerve is practically that of a .745 grammes per cent. solution of potassium chloride.

EXPERIMENT A

Sciatic Nerve of cat immediately removed from the animal after death.

The following measurements were made before and after immersion of the nerve in .745 per cent. KCl ($\frac{1}{10}$ normal solution), for five minutes, at 18° C. :—

Before			After		
Weight200 grammes *	Weight201 grammes *
Length	...	4.7 centimetres	Length	...	4.6 centimetres
Resistance	...	20,500 ohms	Resistance	...	20,100 ohms

* The treatment to which these nerves were subjected was exactly the same as that dealt out to the nerves used in the experiments of the preceding section: the time of immersion, the temperature of the solution, the method of subsequent drying of the nerve in filter paper, etc.

The attempt was always made in the subsequent drying of the nerve to regain after immersion the original condition of the nerve, as far as the presence of surface moisture is concerned. To

succeed in this attempt it was considered necessary to deliberately dry the nerve, even at the apparent risk of injuring it, until it no longer left any damp mark upon the filter paper.

The minuteness of the alteration in weight recorded in experiments A and B is taken as evidence of this attempt.

The point is not unimportant, since the fact which it establishes is of interest from the point of view that the external solution is a 'short circuiting solution.' The fact may be stated as follows :—

The bulk of the pre-existent external solution, unlike its conductivity, was not affected in these experiments.

From these data the 'specific resistance' in each case can be calculated (for method see p. 260) and is—

Before	After
186 ohms	190 ohms

corresponding to a 'specific conductivity' in each case respectively of 50.7 and 50.2, expressed in the usual units.

The alteration in the specific conductivity is therefore extremely small, and may, for the purposes of calculation from the data of the next experiment, be neglected.

EXPERIMENT B

Sciatic Nerve of cat immediately removed from the animal after death.

Measurements taken before and after immersion in a 1.49 grammes per cent. solution of potassium chloride ($\frac{1}{2}$ normal solution), for five minutes, at 18° C.

Before			After		
Weight	...	2035 grammes	Weight	...	1995 grammes
Length	...	5 centimetres	Length	...	5 centimetres
Resistance	...	22,276 ohms	Resistance	...	20,500 ohms
Specific resistance		181 ohms	Specific resistance		164 ohms
Specific conductivity		52.7 in terms of mercury at 18° C $\times 10^{-8}$	Specific conductivity		58.1 in terms of mercury at 18° C $\times 10^{-8}$

In this experiment there is a considerable alteration in the 'specific conductivity.' This increase is also not to be explained by the addition of a new cylindrical covering of solution in excess of its pre-existent one, since the weight has diminished by 4 milligrammes, most probably by the abstraction of water from the internal solution.*

* Preliminary Communication, *Proceedings Royal Society*; 67, 317.

From this fact and from the following considerations an attempt is made to deduce the conductivity of the internal solution.

In the first place, it is assumed that an 'external solution' of the conductivity of .745 grammes per cent. KCl has been replaced by a similar bulk of a solution of 1.490 per cent. KCl, an assumption which is justified by the regularity of the results obtained in the preceding section of this paper.

In the second place, the alteration in weight is taken as too small to affect the result.

It may, therefore, be considered that the following equations represent the conditions present :—

$$\begin{array}{lcl}
 (1) \text{ The conductivity as } & \left. \begin{array}{l} \text{measured before} \\ \text{immersion.} \end{array} \right\} & = \left\{ \begin{array}{l} \text{The conductivity of the pre-existent 'external} \\ \text{solution,' having the same specific con-} \\ \text{ductivity as one-tenth normal solution of} \\ \text{KCl.} \\ \qquad \qquad \qquad + \\ \text{The conductivity of the internal solution.} \end{array} \right. \\
 (2) \text{ The conductivity as } & \left. \begin{array}{l} \text{measured after} \\ \text{immersion.} \end{array} \right\} & = \left\{ \begin{array}{l} \text{The conductivity of a solution of one-fifth} \\ \text{normal KCl, having the same spatial} \\ \text{distribution as the pre-existent 'external} \\ \text{solution.'} \\ \qquad \qquad \qquad + \\ \text{The conductivity of the internal solution.} \end{array} \right.
 \end{array}$$

But these equations are simplified by the fact that the specific conductivities of one-tenth and one-fifth the normal KCl solution are known and are to one another practically as 1 is to 2.

Therefore,

$$\begin{array}{lcl}
 (1) & 52.7 & = C_e + C_i \\
 (2) & 58.1 & = 2 C_e + C_i
 \end{array}$$

where C_e is the conductivity of the 'external solution' and C_i is the conductivity of the 'internal solution,'

\therefore by subtracting (1) from (2)

$$C_e = 5.4$$

That is to say, that the conductivity due to the pre-existent external solution is practically one-tenth of the total conductivity of the nerve.

As a corollary it follows that the conductivity of the axis cylinders accounts for nine-tenths of the total conductivity of the nerve.

In the sciatic nerve of the cat only one-third of the total area of the nerve is taken up by the circular bundles of nerve fibres. This figure was obtained by casting the enlarged shadow of a cross section upon a screen, drawing over the shadow, cutting up and weighing the paper.

Let us, as a concession, admit that one-half of the space is so taken up.

In the nerve bundle only one-third of the space is taken up by the axis cylinders of the nerve fibres. This figure was found by the examination of an enlarged microphotograph of a cross section of a fasciculus of nerve fibres.*

On this computation only one-sixth of the total cross section of the nerve consists of cross sections of axis cylinders.

Let us, as a further concession, admit that the axis cylinders form one-fifth of the total bulk of the nerve.

Therefore, we find ourselves to have come to the opinion that structures occupying only one-fifth of the bulk of the nerve account for nine-tenths of its electrical conductivity.

The amount of water in the nerve is not more than two-thirds of its weight. All the electrolytes which can conduct an electrical current are in solution in this water.

Let us suppose the water to be uniformly distributed throughout the nerve trunk, to be all free to take electrolytes into solution, and admit that two-thirds of the mass of the axis cylinders consists of solutions of electrolytes.

Then it follows that solutions occupying only two-fifteenths—

$$\frac{2}{3} \times \frac{1}{5} \quad \text{or} \quad \frac{2}{15} \text{ ths}$$

of the total bulk of the nerve account for nine-tenths of its conductivity.

Truly, although the total conductivity of the nerve is small, the specific conductivity of these solutions in the axis cylinders of the nerve must be very great.

$$\text{The specific conductivity of nerve} = 50 \quad \dots \quad (\text{Hg.} \times 10^{-8}).$$

$$50 \times \frac{9}{10} = 45$$

The specific conductivity of solutions occupying only 2-15ths of the space, and accounting for a conductivity of 45 is equal to

$$4 \times \frac{15}{2} \text{ or } 340 \text{ (approx.)}$$

That is to say, upon this computation, that the solutions of the axis cylinder have a conductivity as great as that of a 2.6 grammes per cent. solution of KCl.

These figures are large. They are not so large as an admission of any, even of the necessary, imagination might have made them. They are too small to explain the facts of the previous section, but even in this form they are large enough to provide a basis for criticism of the 'apparently' concentrated solutions of the axis cylinder.

* This figure was determined by the examination of a square area of the drawing in Böhm-Davidoff and Huber; p. 143.

GENERAL CONCLUSIONS

It is impossible, in considering the electrical phenomena accompanying manifestations of change in the body, to neglect the primary importance of processes of diffusion in their production: and in no case is such a statement more apposite than when it is brought to bear upon electrical phenomena determined by injury. For the quantitative distribution of electrolytes in the tissues is notoriously by no means uniform, and the tendency to uniformity which follows injury is necessarily the cause of their redistribution.

It, therefore, follows that in the case of any remarkable electrical phenomenon, attributable to 'injury,' the part taken in its production by this redistribution of electrolytes must *necessarily* be examined. Where this enquiry has not been elaborately made, there is reason to undertake it, even if some other cause has, upon apparently adequate grounds, been previously assigned to the phenomenon.

When, as in the case of the injury current of nerve, the pursuit of the cause has been abandoned, and an agreement has been come to, to cover the abandonment by a phrase; then, such a course can only be justified upon the grounds that the phenomenon is of very minor importance, and is better disregarded whilst more fundamental facts are being observed and investigated.

In the case of the injury current of nerve the abandonment has been definite, the justification has not been pleaded; since under whatever phrases the phenomenon and its causation have been concealed, it is a matter of common opinion that this phenomenon may be a crude, stationary, and therefore useful, instance of the travelling phenomenon of the nervous impulse.

The lack of justification is not only made evident by such an important consideration, but even better so by another still more important one; for the evidence is by no means conclusive, is even fallacious when apparently most definite, which has been used to prove that the injury current is not the outcome of conditions pre-existing in the nerve fibre.

As long as it remains possible to regard this phenomenon as due to previously existent structures newly arranged in regard to one another by the process of injury; so long must it be regarded as probably a most important guide to the differences of structure between the component parts of the nerve; since such differences can be estimated by use of the electrical phenomenon as an index, and the new arrangement consequent upon injury is capable of being directly studied.

There is no justification, therefore, for an abandonment of the enquiry into the causation of this phenomenon.

There is one 'pre-existent structure' of the nerve which necessarily plays an important part, namely, the barrier (or barriers) which previously to the occurrence of 'injury' had maintained separate and distinct from one another the different structures of the nerve.

There is another 'pre-existent structure' also of undoubted importance in determining the value and direction of the phenomenon, namely, the solution which bathes the outer surfaces of the nerve fibres.

The value of this last factor has been studied in this paper, and it has in this research been definitely proved to have a value which is only and completely given to it by the fact that it is a solution of electrolytes : a value which may be altered in a precise and quantitative manner by modifications of the electrolytes which it contains ; these modifications adding to it, subtracting from it, and even reversing it.

The strength of the solution, as it exists upon the nerve removed from a living animal or immediately after death (before the stoppage of the circulation has had time to lead to local modifications in the lymph), is that of the ordinary 'normal saline' solution ; that is to say it is 'entirely pre-existent : ' modifications occurring later are only sources of error in the estimation of its importance.

The remaining structure upon which the injury current depends, the 'internal solution' of the nerve, as necessarily owes all its importance and value to the fact that it is a solution of electrolytes ; and it is, as such, capable of modification by the addition or subtraction of electrolytes.

The decision as to its 'pre-existent' or 'newly acquired' importance, as determining the value of the injury current, lies entirely in the answer to this question.

Are the electrolytes in this solution, which render its value (as a solution) different from that of the external 'normal saline,' pre-existent ; or are they newly contributed by chemical change, the secondary consequence of injury ?

It is considered that this question is best approached in the following way.

A study of the polarization phenomena of nerve has led to an appreciation of the fact, that the greater part of the conductivity of the nerve is due to the internal solutions of the axis cylinders.

If this greater conductivity means a greater specific conductivity of these solutions, then the injury current is explained in terms entirely of pre-existent structures. If it does not, but means a greater volume of 'normal saline' solution within the axis cylinder than outside of it in the nerve trunk, then the axis cylinder solutions form by far the greatest mass of conducting structure in the nerve.

Changes in the specific conductivity of this mass, such as would follow the addition of electrolytes by chemical change affecting each unit of it, and subsequent to injury, must necessarily add considerably to the general conductivity of the nerve.

There is, however, no evidence that the conductivity of the nerve is affected by injury other than such as is adequately explained by the destruction of 'membranes,' structures which are characterized by low specific conductivity. There is, therefore, no evidence of the addition of electrolytes to the internal solution, nor even of the addition of electrolytes to localized portions of this solution. There is, therefore, no evidence that secondary chemical change takes any part in the development of the injury current.

There is, on the contrary, evidence that all the conductivity of nerve is adequately explained by the presence of its inorganic salts, and, therefore, that all the electrolytes of normal nerve are inorganic salts. The electrical phenomena of nerve, if such evidence is considered as conclusive, depends entirely upon the inorganic salts which it contains.

If it is proven that the injury current is due to a 'pre-existent' differential distribution of electrolytes, and that all the electrolytes it contains are its organic salts, then the injury current becomes disappointingly a guide to nothing more than the differential distribution of inorganic salts in the nerve.

The disappointment is modified, however, when the extraordinary nature of the differential distribution, as indicated by this phenomenon, is realized. For the indication it gives is, that there is to be found within the axis cylinders a solution of extraordinary concentration; a possibility itself intrinsically of great interest.

When such a discovery is considered solely from the point of view of the previously determined facts of the conductivity of nerve, and the secondary consequences of conduction by a nerve (the electrotonic phenomena), then it appears at once as the necessary corollary to such facts. Indeed, the probable occurrence of such a differential distribution of electrical conductors within the nerve, as indicated by these facts, has often been considered, even if no definite limits have been given to the speculation. The results of other methods of investigation used by physiologists may be said to have determined the expectation of such a condition, which expectation these results have amply justified.

When such a differential distribution is, however, considered from another side, there is a very grave difficulty in accepting its possibility. For such a concentrated solution placed within the cell of an osmometer, separated only by a semi-permeable membrane from the dilute 'normal saline,' would be found capable of giving rise to a pressure of many atmospheres inside the cell. There is no knowledge available to decide the possible magnitude of the strain, which the extremely minute capillary tubes, in which we may presume this solution lies, would stand; but it is, in ignorance, inconceivable that they should stand such a strain as this.

On the other hand, there is reason to believe that in such capillary tubes the expected osmotic pressure may not arise, although highly concentrated solutions are present. Thus, there is the fact that fibrillar structures may actually concentrate

within their pores solutions in which they are immersed, so that the pores finally contain solutions much more concentrated than the 'mother' solution surrounding the fibrillar structure. Such a fact is extremely suggestive when the extremely fine nature of the interspaces which lie between the fibrillae of the axis cylinder is considered. It is more suggestive still when the possibility of regarding the fibrillae as themselves tubular is taken into account.

Accepting all that is taken as known of the minute microscopical structure of the axis cylinder of the nerve: then there is no inherent improbability in the supposition that the inorganic salts of the nerve might there be held enchained in a highly concentrated solution free to move parallel to, but not at right angles to and away from the fibrillae.

Granting such a possibility, we are, however, faced by the important corollary that such concentrations are indeed enchained there, and are, therefore, unable to exert an osmotic pressure, or by diffusing away give rise to electrical phenomena. To explain, in the presence of such an hypothesis, diffusion processes consequent upon injury, it seems necessary to invent a phenomenon really secondary to the injury, involving new conditions of the fibrillar structure.

To invent such a phenomenon is as culpable as the invention of a chemical change, and the necessity for doing so is equivalent to the necessity for abandoning this supposition.

On no lines known, therefore, can we explain how we could place and retain in, and subsequently allow to diffuse away from the axis cylinders, a highly concentrated solution of electrolytes. But the physical capabilities of such a position, in the longitudinal pores of capillary tubules so minute, are unknown, and beyond the reach of investigation.

Granted strong presumptive evidence of the presence of a highly concentrated solution, and of the possibility of its diffusing away from it subsequently to an 'injury,' it is essential first to criticize severely the nature of the evidence.

Granted that the evidence is found satisfactory, then the question of possibilities may be with greater advantage discussed; since such a case might in itself light up possibilities.

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OBSERVATIONS ON THE PHYSIOLOGY OF THE
CEREBRAL CORTEX OF SOME OF THE
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OBSERVATIONS ON THE PHYSIOLOGY OF THE CEREBRAL CORTEX OF SOME OF THE HIGHER APES

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We have been engaged for some time past on inquiry into the physiology of the cerebral cortex of the anthropoid apes. We are able to lay before the Society some new facts regarding the topographical distribution of function in the anthropoid brain. Our experiments have been carried out on individuals representing the four species *Pithecus satyrus* (Orang), *Troglodytes gorilla* (Gorilla), *Troglodytes niger* (Chimpanzee), and *Troglodytes calvus* (Chimpanzee). The specimens so far have included ten adult individuals. Of *Troglodytes niger* one individual used was only a few months old.

I. METHOD EMPLOYED

The method of excitation employed for the cortex has been unipolar faradization, in the manner previously adopted by one of us¹ in examining the cortex cerebri for ocular reactions. This method allows of finer localization than that possible with the double-point electrodes ordinarily used. The inductorium (Kronecker's pattern and scale) has been Helmholtzed.

II. 'MOTOR' (SO-CALLED) AREA

This area we find to include continuously the whole length of the precentral convolution. It also enters into the whole length of the *sulcus centralis*, with the usual exception of its extreme lower tip and its extreme upper tip.

1. Sherrington, *Roy. Soc. Proc.*, vol. 52, 1893.

In all the animals examined, we have found the 'motor' area not to at any point extend behind *sulcus centralis*. Feeble reactions can occasionally, under certain circumstances, be provoked by strong faradization behind the *sulcus centralis*, but these are equivocal, and appear under conditions that exclude their acceptance as equivalent to 'motor-area' reactions.

On the mesial surface of the hemisphere the 'motor' area has extended less far down than was expected. It has not extended to the calloso-marginal fissure. Certain areas near that fissure have yielded us movements, *e.g.*, of shoulder, body, wrist, and fingers; but we hesitate, for reasons to be given in a fuller communication, to class these with those of the 'motor' area proper.

We have found the precentral convolution excitable over its free width, and continuously round into and to the bottom of the *sulcus centralis*. The 'motor' area extends also into the depth of other fissures besides the Rolandic, as can be described in a fuller communication than the present. The hidden part of the excitable area probably equals, perhaps exceeds, in extent that contributing to the free surface of the hemisphere. We have in some individuals found the deeper part of the posterior wall of the *sulcus centralis* to contribute to the 'motor' area.

In the 'motor' area we have found localized, besides very numerous other actions, certain movements of the ear, nostril, palate, movements of sucking, of mastication, of the vocal cords, of the chest wall, of the abdominal wall, of the pelvic floor, of the anal orifice, and of the vaginal orifice. We have met with various examples of inhibition effects produced by this cortex, such as described by one of us previously in the cortex of the lower apes.¹

We find the arrangement of the representation of various regions of the musculature follow the segmental sequence of the cranio-spinal nerve-series to a very remarkable extent. The accompanying figure indicates better than can a verbal description the degree of adherence to this sequence.

We do not find that the exciting current for the 'motor' cortex requires to be extremely strong for the anthropoid brain. 'Epilepsy' is easily evoked from the cortex of the anthropoids.

Our experiments show that the *sulci* in the region of cortex dealt with can in no sense be considered to signify physiological boundaries. Further, the variation of the *sulci* in these higher brains is so great from individual to individual that, as our observations show, they prove but precarious, even fallacious, landmarks to the details of the true topography of the cortex.

[The mere fact that the 'motor' area extends in front of but never (so far as our experiments have yet gone) behind the *sulcus centralis*, is but little indication of detailed constancy of relation between the physiological area and even that sulcus,

1. Sherrington, *ibid.*, also Sherrington and Hering, *ibid.*, vol. 62, 1897, and Hering and Sherrington, *Pflügers Archiv.*, vol. 71, 1897.

though such a fundamental one ; the antero-posterior diameter of the sulcus, being an area often five-and-twenty millimetres across, it is, when treated as marking a line on the cerebral surface, but a rough guide for any detailed examination of the functional topography.]

Extirpation of the hand area by itself has been followed by severe paresis of the hand, the hand being for a few days practically useless and seemingly powerless. In a few weeks use and 'power' were remarkably regained in the hand, so that it was once more used for climbing, etc. The animal ultimately not unfrequently fed itself with fruit, making use of that hand alone. Even small ablations in the precentral gyrus have led to severe though quickly diminishing pareses. On the other hand, ablations of even large portions of post-central gyrus have not given any even transient paresis.

III. OTHER REGIONS OF CORTEX

Our observations indicate that the frontal region, yielding conjugate deviation of the eyeballs, presents such marked differences of reaction from the 'motor' area of the Rolandic region that we hesitate to include it with the so-called 'motor' cortex ; it seems necessary to distinguish it in a physiological category separate from that. Spatially it is wholly separated from the Rolandic 'motor' area by a field of 'inexcitable' cortex.

As to the occipital lobe, only from the extreme posterior apex of the lobe and from its actual calcarine region has faradization yielded any movement (eyes), and then not easily.

We hope at no long distance of time to be able to lay before the Society a detailed account of the completed investigation. Some of our experiments are still in progress.

It is a pleasure to record here our indebtedness to Dr. L. MOND, F.R.S., for enabling us to bring these experiments to their present stage.

ADDENDUM ON THE PYRAMIDAL TRACTS

BY C. S. SHERRINGTON

The spinal degeneration resulting from ablation in the precentral gyrus of the above-mentioned 'hand'-area, discovers in the anthropoid cord the human feature of a perfectly large direct ventral (Türcksbündel) as well as crossed pyramidal tract. The relative sizes of these tracts seem about the same as in man.

The homolateral or uncrossed lateral division of the pyramidal tract is also well seen. The crossed pyramidal degeneration from the hand area lesion is clearly traceable down to the lumbar region of the cord. In the lowest brachial segments there is obvious degeneration of fibres in the grey matter of the ventral horn of the crossed side. Some of the large nerve-cells there seem also degenerate.

A lesion at the top of the *gyrus precentralis* gave no ventral pyramidal tract degeneration, and only a very slight uncrossed lateral pyramidal, although an extensive crossed lateral. that descends the whole length of the cord.

TUBERCULAR EXPECTORATION IN PUBLIC
THOROUGHFARES

TUBERCULAR EXPECTORATION IN PUBLIC THOROUGHFARES :

AN EXPERIMENTAL INQUIRY

By H. E. ANNETT

No greater stimulus could have been given to a renewed and more careful study of the etiology of tuberculosis of man and animals than the astounding announcements made by ROBERT KOCH at the Meeting of the British Congress on Tuberculosis,¹ held in London on July 22, 1901. Whether his chief statements, over which much controversy has arisen, viz. :—

1. 'That human tuberculosis differs from bovine, and cannot be transmitted to cattle' ;
2. 'That it is not the case that many cases of tuberculosis are caused by the consumption of alimenta containing tubercle bacilli among the inhabitants of large cities, especially the children' ;
3. 'That although the important question as to whether man is susceptible to bovine tuberculosis at all is not yet settled, one is at liberty to say that, if such a susceptibility really exists, the infection of human beings is but a very rare occurrence.'

be right or wrong, it is certain that at the present time extreme energy is being exerted by bacteriologists and hygienists to confirm or negative the results of his extensive experimental work² on the subject.

However, at the present stage, in spite of the results obtained by this illustrious author, and of his 'belief that it is not advisable to take measures against the infection of man by the milk and flesh of tuberculous cattle,' it would be somewhat hazardous to relax the sanitary control of the milk supplies of our large towns—a control which is just becoming firmly established.

Particular attention is naturally directed to investigations into the methods by which infection may be brought about by human tubercular sputum, and to the measures which might be employed to prevent such infection ; for all observers agree with KOCH that human sputum is the main source of human tuberculosis :

1. *British Medical Journal*, July 27, 1901, p. 191.

2. *Arch. f. wissenschaft. u. prakt. Theiirkeilkunde*, 1902, XXVIII, p. 169.

'As to the question where the inhaled tubercle bacilli come from there is no doubt; certainly they get into the air with the sputum of consumptive patients. By coughing, and even speaking, sputum containing bacilli is flung into the air in small drops, and can at once infect persons who happen to be near the cougher. But it may also be pulverized, when dried, in the linen or on the floor, and get into the air in the form of dust.'

Although it is certain that the sputum expectorated by tubercular individuals is most commonly a very dangerous source of infection in the overcrowded and ill-ventilated dwellings, workshops, and other rooms occupied by the lower classes; still, there can be but little doubt that infection may arise from the inhalation of dust particles in our public thoroughfares and streets, and that this will continue a source of public danger until an efficient means of prevention can be inaugurated.

BROUARDEL at the same Congress characterized the custom 'of expectorating on the ground as a disgusting and dangerous habit,' and stated 'that once the habit has disappeared, tuberculosis will decrease rapidly.'

Tubercle bacilli in sputum expectorated on the street walks are influenced there by two physical agencies—desiccation and the action of light—and also are submitted to any influence which may be exerted by putrefactive and other organisms in the sputum or gaining access to it. It is well known that tubercle bacilli resist the action of such organisms for a long period, and in general the expectorated mass has become a powdered dust before their action is likely to exert any deleterious effect on the tubercle bacilli.

The action of desiccation has been known for many years. SCHILL and FISCHER shewed that tubercle bacilli in sputum, dried in a thin layer, retained their virulence for six months; while SORMANI shewed that sputum dried in a thin layer on glass was not virulent after four months. CORNET¹ believes that, considering that tubercle bacilli may continue virulent in the dust of dried sputum for at least three or four months, in the country, where the dust is diluted by a large volume of air, the chance of infection thereby is but small; but in large towns, particularly in the most frequented streets in summer or during continued dry weather, the infecting material becomes more abundant, and there is danger of infection with the tubercle bacilli in street dust.

Experiments by NUTTALL² seem to indicate that under favourable temperature circumstances tubercle bacilli may multiply in tubercular sputum outside the body.

There now remains to be considered only the action of light on the tubercle bacilli in sputum expectorated on to the street sidewalks. KOCH³ states that 'he was able to determine that tubercle bacilli, according to the thickness of the layer in which they were exposed to sunlight, were killed in a few minutes to some hours. Also,

1. Cornet, *Zeitsch. Hygiene*, vol. V, p. 288.

2. Sternberg, *Manual of Bacteriology*, p. 381.

3. Koch, *Verhandlungen d. Internatl. Congr., Berlin, 1890*, p. 142.

that diffuse daylight had a similar effect; cultures of tubercle bacilli die if they are exposed close to the window for five to seven days.'

FELTZ asserted that pulverized tubercular sputum exposed to sunlight was virulent after one hundred and forty days. MIGNECO,¹ as the result of a number of experiments, makes the following conclusions:—

1. That sunlight has an injurious effect on *Bacillus tuberculosis* as upon other organisms.
2. That tubercle bacilli contained in sputum on soiled linen and woollen stuffs do not withstand the action of sunlight for as long a period as twenty-four to thirty hours—if the layer of sputum be not too thick.
3. The virulence of the tubercle bacilli gradually diminishes after ten to fifteen hours of exposure to sunlight, and after this period is lost.

RANSOM and SHERIDAN² shewed that tubercle bacilli in sputum and in culture, and also in dry and finely divided material exposed to the action of light and air, quickly lose their virulence.

SAWIZKY³ undertook two sets of experiments, in one of which tubercular sputum was dried and preserved in a dark place; in the other the sputum was exposed for various periods to the action of sunlight. His results shew:—

1. That in ordinary living rooms, dried tubercular sputum retains its virulence for a period of two and a half months.
2. The virulence of such a sputum is not suddenly, but is gradually, lost.
3. Dried tubercular sputum exposed to action of direct sunlight loses its virulence similarly to sputum kept in the dark.

The results of the investigations of these different authors on the action of light on tubercle bacilli in sputum thus appear to be somewhat at variance, and the action of light on the organisms in sputum expectorated on the sidewalks of public thoroughfares under all the varying conditions of wind, sunshine, and rain of an English climate, still requires investigation. This will form the subject of a subsequent communication.

The occurrence of tubercular sputa among the many masses which testify to 'the disgusting and dangerous habit' of expectorating in the public thoroughfares of all large towns is illustrated in the following table. The expectorations were collected during the winter months, by means of sterilized swabs and placed in sterilized test tubes. Portions of each were inoculated subcutaneously into two guinea pigs, which were kept under observation during the subsequent eight weeks or more. Other portions were used to make two smears from each on glass slides, which after being stained by ZIEHL-NIELSEN's method were examined under a one-twelfth O.I. objective. Mucous, muco-purulent, and purulent accumulations were taken indiscriminately, only the most liquid expectorations being neglected.

1. Migneco, *Arch. f. Hygiene*, XXV, p. 361.

2. Ransom and Sheridan, *New York Medical Journal*, 1894, No. 12.

3. Sawizky, *Inaug. Dissertn.*, St. Petersburg, 1891; *Centr. f. Bakt.*, 1892, p. 153.

TABLE OF EXPERIMENTS

No.	Collected in			Appearance	Result of Microscopical Examination	Result of Inoculation into Guinea Pigs
1	Hanover Street	Muco-purulent	No tubercle bacilli	No tuberculosis
2	"	"	" "	" "
3	"	Mucous	" "	" "
4	"	Muco-purulent	" "	" "
5	Church Street	"	" "	" "
6	Whitechapel	Mucous	" "	" "
7	Crosshall Street	Muco-purulent	A few tubercle bacilli	Both animals killed after eight weeks: local and general tuberculosis
8	"	Mucous	No tubercle bacilli	No tuberculosis
9	"	Muco-purulent	" "	" "
10	"	"	" "	" "
11	"	Mucous	" "	" "
12	"	Muco-purulent	" "	(1) Died in eight days—Diplococcal Septicaemia
13	Dale Street	Purulent	" "	No tuberculosis
14	"	"	" "	" "
15	Pembroke Place	Muco-purulent	" "	" "
16	Brownlow Hill	"	" "	" "
17	"	"	" "	" "
18	Lime Street	"	" "	" "
19	St. Anne Street	"	" "	" "
20	"	"	" "	" "
21	"	"	" "	" "
22	"	Purulent	" "	" "
23	"	Muco-purulent	Tubercle bacilli present	Both animals developed local and general tuberculosis
24	Smithdown Road	"	No tubercle bacilli	No tuberculosis
25	"	Mucous	" "	" "
26	Upper Parliament Street	Muco-purulent	" "	" "
27	"	"	...	"	" "	" "

TABLE OF EXPERIMENTS—*continued*

No.	Collected in			Appearance	Result of Microscopical Examination		Result of Inoculation into Guinea Pigs	
28	Myrtle Street	Purulent	No tubercle bacilli		(1) Died in two days, (2) Died in four days (Diplococci in heart's blood)	
29	Bold Street	Muco-purulent	„	„	No tuberculosis	
30	„	Mucous	„	„	„	„
31	„	Muco-purulent	„	„	„	„
32	Church Street	Purulent	„	„	„	„
33	„	Muco-purulent	„	„	„	„
34	Whitechapel	„	„	„	„	„
35	Myrtle Street	„	„	„	„	„
36	Renshaw Street	„	„	„	(1) „ „ (2) Died in ten days	
37	Parker Street	Mucous	„	„	No tuberculosis	
38	Great Charlotte Street	„	„	„	„	„
39	Queen's Square	Muco-purulent	„	„	„	„
40	William Brown Street	„	„	„	„	„
41	London Road	Purulent	„	„	„	„
42	Pembroke Place	Muco-purulent	„	„	„	„
43	Crown Street	„	„	„	„	„
44	„	„	„	„	„	„
45	„	„	„	„	„	„
46	Earle Road	„	„	„	„	„
47	Durning Road	„	„	„	„	„
48	„	„	„	„	„	„
49	„	Mucous	„	„	„	„
50	Holt Road	Muco-purulent	„	„	„	„
51	Kensington	„	„	„	„	„
52	„	„	„	„	„	„
53	Prescott Street	„	„	„	„	„
54	Myrtle Street	„	„	„	„	„
55	Lime Street	Mucous	„	„	„	„

TABLE OF EXPERIMENTS—*continued*

No.	Collected in			Appearance	Result of Microscopical Examination	Result of Inoculation into Guinea Pigs
56	Lime Street	Muco-purulent	No tubercle bacilli	No tuberculosis
57	London Road	Mucous	" "	" "
58	Falkner Street	Muco-purulent	An occasional tubercle bacillus	Both animals developed local and general tuberculosis
59	"	"	No tubercle bacilli	No tuberculosis
60	"	Purulent	" "	" "
61	Crown Street	"	" "	" "
62	Myrtle Street	Muco-purulent	" "	" "
63	"	"	" "	(1) Died in six days (2) No tuberculosis
64	"	"	" "	No tuberculosis
65	Hardman Street	"	" "	" "
66	Bold Street	Purulent	" "	" "
67	Paradise Street	Muco-purulent	" "	" "
68	Hanover Street	Mucous	" "	" "
69	Dale Street	Muco-purulent	" "	" "
70	"	"	" "	" "
71	"	"	" "	" "
72	"	"	" "	Both developed local and general tuberculosis
73	William Brown Street	...		"	" "	No tuberculosis
74	"	"	...	"	" "	Both developed local and general tuberculosis
75	London Road	"	" "	No tuberculosis
76	"	"	" "	" "
77	Castle Street	"	" "	" "
78	Tithebarn Street	"	" "	" "
79	"	"	" "	" "
80	Hatton Garden	"	" "	" "
81	Dale Street	"	" "	" "
82	Byrom Street	"	" "	" "

TABLE OF EXPERIMENTS—*continued*

No.	Collected in			Appearance	Result of Microscopical Examination		Result of Inoculation into Guinea Pigs
83	Great Howard Street	...		Purulent	No tubercle bacilli		(1) Died in five days—diplococci in heart's blood (2) No tuberculosis
84	Dale Street	Muco-purulent	"	"	No tuberculosis
85	"	"	"	"	(1) Died in three days (2) No tuberculosis
86	Hardman Street	"	"	"	(1) No tuberculosis (2) Died in eight days
87	Bold Street	Purulent	"	"	No tuberculosis
88	Old Hall Street	Mucous	"	"	" "
89	Hardman Street	"	"	"	" "
90	Bold Street	Muco-purulent	"	"	" "
91	"	"	"	"	" "
92	Hanover Street	"	"	"	(1) No tuberculosis (2) Died in four days
93	"	"	"	"	No tuberculosis
94	Paradise Street	Purulent	"	"	" "
95	Great Howard Street	...		Muco-purulent	"	"	(1) Died in five days—extensive oedema (2) No tuberculosis
96	" "	...		"	"	"	No tuberculosis
97	Lime Street	"	"	"	" "
98	St. Anne Street	"	"	"	(1) Died in five days (2) No tuberculosis
99	"	Purulent	"	"	No tuberculosis
100	Great Howard Street	...		Muco-purulent	"	"	" "
101	" "	...		"	"	"	Both animals died in five and eight days respectively
102	Tithebarn Street	"	"	"	No tuberculosis
103	"	"	"	"	Both died in a few days
104	Upper Parliament Street	...		"	"	"	No tuberculosis
105	"	"	...	"	"	"	(1) Died in seven days—diplococci in heart's blood (2) No tuberculosis
106	"	"	...	"	"	"	No tuberculosis
107	Myrtle Street	"	"	"	" "
108	"	Mucous	"	"	" "

Thus, neglecting those cases which resulted in the death of both experimental animals within a few days, out of one hundred and five sputa collected, five were proved to contain virulent tubercle bacilli, a percentage of 4.76; three of the five being demonstrated microscopically. Some of the collections were certainly nasal secretions, discharged by way of either the anterior or posterior nares.

The number of these sputa expectorated on to the side walks of the principal streets of large towns during the day reaches a large figure. I tried to count all those visible between ten and eleven o'clock on the morning of March 1, 1902, during a slow walk occupying one hour, along the most frequented streets of Liverpool; but the actual expectorations were far too numerous to count; so that, omitting all very liquid ones, note was taken of all which appeared to have been recently expectorated, and which consisted of more or less thick mucous, mucopurulent, or purulent discharges.

Brownlow Hill : part between University College and Lime				
Street—sunny side only	72
Ranelagh Street—shaded side	14
Church Street—sunny side	10
Lord Street	„	11
Castle Street	„	7
Dale Street—shaded side	30
William Brown Street—sunny side	5
London Road—shaded side	18
Pembroke place	„	16

These figures give only a very faint idea of the extent of the expectoration which is going on each hour in streets, and consequently of the extent to which pedestrians are subjected to infection by tubercle bacilli.

Besides the possibility of the masses drying in a few hours and of the bacilli being carried and thrown about in the dust of the air, sputum also soils the boots of passers by, but more particularly the trailing edges of ladies' skirts; tubercle bacilli becoming freely distributed into offices, shops, dwelling-houses, etc., by this means.

It is evident, therefore, that the indiscriminate expectoration of tubercular sputum into public thoroughfares, and particularly on the side walks of streets, is a danger (the extent of which cannot be at present exactly defined) to the public health, and measures for its prevention must be considered by sanitary authorities.

The measures, which might be adopted, are :—

1. The education of the public as to the dangers which arise from indiscriminate expectoration, not only in dwelling-houses, but also in public places.
2. Regulations and Byelaws.

Through the courtesy of Surgeon-General W. Wyman, of the United States Marine Hospital Service, and of Surgeon G. Purviance and Assistant-Surgeon J. F. Anderson, I have been able to obtain the Regulations in force in the large towns of the States.

BALTIMORE :—

‘Be it enacted and ordained by the Mayor and City Council of Baltimore, that it shall not be lawful for any person to spit or expectorate upon the floor of any street car or public conveyance, or of any public building within the City of Baltimore, under a penalty for each and every offence of a fine of one dollar, to be recovered as are other fines for the violation of city ordinances.’

WASHINGTON :—

‘That it shall be unlawful for any person to expectorate or spit on any part of any street railway car, or other public vehicle carrying passengers for hire, or in or upon any part of any public building under the control of the Commissioners of the District of Columbia. Street railway companies, and the proprietors of other public vehicles carrying passengers for hire, shall keep posted conspicuously in each and every one of their cars and public vehicles notice forbidding such expectorating or spitting.

‘Every person as aforesaid violating any of the provisions of any section of this article wherein a penalty is not provided shall, on conviction, be punished by a fine of not less than one dollar nor more than forty dollars for each offense.’

BOSTON :—

‘The Board of Health hereby adjudges that the deposit of sputum in public places is a nuisance, source of filth, and cause of sickness, and hereby orders : that spitting upon the floor, platform, or steps of any railroad or railway station, or car, or from any electric car while said car is in the subway, or elevated above the surface of the ground, or upon the floor, platform, or steps of any public building, hall, church, theatre, market, or any sidewalk immediately connected with said public places, be and hereby is, prohibited.’

NEW YORK :—

Spitting upon the floors of public buildings and of railroad cars and of ferry boats, and upon any station, platform or stairs of elevated railroads, is hereby forbidden, and officers in charge or control of all such buildings, cars, platforms, stairs, and boats, shall keep posted permanently in each public building and each railroad and on each station platform of elevated railroads, and in each ferry boat, a sufficient number of notices forbidding spitting upon the floors, and janitors of buildings, conductors of cars, and employes upon ferry boats and station platforms shall call the attention of all violators of this ordinance to such notices. And it shall be the duty of all persons or corporations manufacturing cigars or conducting the business of printing, where ten or more persons are employed on the premises in the City of New York to provide, and they are hereby required to provide, proper receptacles for spitting, in proportion of one to every two persons employed by them, and that said receptacles be disinfected and cleaned at least once during each working day. That a copy of the second paragraph of this section be kept posted permanently in a conspicuous place in all cigar manufactories and in printing offices where ten or more persons are employed.’

From these extracts it is seen that up to the present legislation has been directed almost solely towards the prevention of the habit of expectorating chiefly in public buildings and public conveyances. In Liverpool and many of the other large towns in England the only attempts made to prevent the habit consist simply in the posting of notices prohibiting spitting in tramcars and public buildings. The present powers of local authorities not permitting of the breach being treated as a punishable offence, these notices are largely neglected. There can be no doubt, in the light of the results of the above experimental investigation, that more strict measures must be adopted by sanitary authorities to prevent expectoration, not only on public conveyances and buildings, but also in public thoroughfares.

3. A further measure might be adopted by sanitary authorities, of the daily washing down and cleansing of the sidewalks of the principal streets. In many towns the paved roadways are regularly flushed. A similar procedure practised on the sidewalks would effectually get rid of all sputum, and, moreover, remove the excreta of dogs and other disgustingly undesirable material which soil the public footpaths of our large towns.

PSEUDO ACTINOMYCES OF THE UDDER OF
THE COW

PSEUDO ACTINOMYCES OF THE UDDER OF THE COW

By RUBERT BOYCE

The following case is interesting from several points of view. The cow, from which the specimens were taken, was thought to be suffering from tubercle of the udder, but it failed to react to the tuberculin test although the tuberculin was active and had caused reactions in other animals proved to be suffering from tubercle. Secondly, inoculation experiments of the diseased tissue into guinea pigs failed to demonstrate the presence of tubercle. Thirdly, histological examination revealed nodules in the mamma composed of proliferating connective tissue cells, lymphoid cells and leucocytes, but no giant cells nor tubercle bacilli; on the other hand, there were typical mulberry-like masses which recalled at once the appearance of *Actinomyces*, and led me at first to consider the case as one of actinomyces of the udder of the cow. The failure, however, to produce any reaction in the guinea pig, and the absence of any definite hyphae, caused me to make a more close examination, and to doubt the fungus nature of the nodules in spite of the presence of the well-marked clubs. Having already had occasion to study the fungus forms met with in the white and black varieties of Madura Foot, in which the nature of the parasite may be altogether masked by deposits, I did not abandon the parasitic theory of the nodules until I had failed with those reagents, which had cleared up the structure of the white and black nodules in Fungus Foot. My failure with these further investigations led me to conclude that the nodules might only be crystalline formations simulating in appearance the Ray Fungus. I was supported in this idea by the fact that whilst Pathologist at the Royal Infirmary I had had referred to me certain portions of diseased breast tissue which had been removed by Mr. PAUL, and which the latter had considered to be examples of Ray Fungus in the human breast. My observations then led me to conclude that in these particular cases the fungus-like appearance was due to a fatty compound. Professor LOESCH has also described, under the head of Pseudo-actinomycosis, brittle, stellate, mulberry masses in the sputa of cases of Pneumonia. Although simulating the Ray Fungus, he regarded them of the nature of leucin. More recently STOELTZNER* has drawn attention to curious crystalline masses, which he found in Rickety bone, and which SALKOWSKI considered closely resembled Spermin in composition.

* *Berliner Klinischer Wochenschrift*, April 30, 1901.

HISTORY OF COW

For the following history I am indebted to Mr. EATON JONES, Veterinary Superintendent of the Liverpool Corporation, who called me in to examine with him the cow, and to witness the *post-mortem*.

‘On October 29, 1901, I examined the animal and found her to be exhibiting all the clinical symptoms of being affected with Tuberculosis, both general and of the udder. She was an aged cow (about nine or ten years), in an emaciated condition, hide-bound, her coat harsh and dry, and suffering from a husky cough, in addition to having chronic induration of the near hind mammary gland. Her past history, as far as I can gather, is as follows:—

‘The man bought her about last June, and she was then in a fairly good condition; but he noticed a small enlargement on the near hind mammary gland about the size of a pigeon’s egg, which gradually grew larger until it had attained the size of a cocoanut. This enlargement on manipulation was of a peculiar nodulated character resembling a bunch of nuts.

She was a good feeder, but despite this, rapidly lost flesh, always had a cough, her faeces were hard and dry, and ordinary purgatives had no effect upon her.

During the time she was in the shippoon she gave about three gallons of milk a day, the milk being very hard to draw, giving an equal quantity from the affected gland, which was likewise of the same colour and consistency as ordinary milk.’

POST-MORTEM EXAMINATION

No tuberculosis of the peritoneum or pleura.

Spleen normal.

Kidney normal.

No enlarged lymphatic glands.

Lungs free from any thickenings or nodular deposits with the exception of a hard calcified area of about the size of a pea.

Udder. Scattered in the substance of the lower portion of the breast tissue were some dozen hard nodules of the size of small filberts, and which during life felt like hard enlarged lymphatic glands. Cut into they were clearly differentiated from the secreting tissue, and presented in their interior small dirty yellow irregular masses, which were hard and gritty to the knife.

From the necrotic looking material in the centre of these masses, coverslip preparations were at once made with a view of demonstrating the suspected tubercle bacilli, none were, however, found; but in order to make certain, inoculations were made.

With the exceptions of these nodules all the organs appeared healthy.

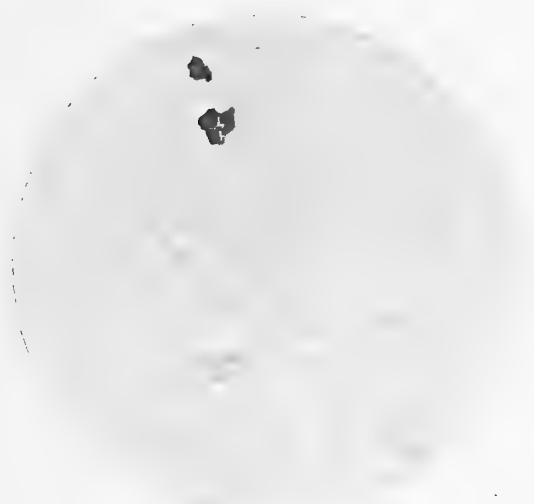


FIG. 1

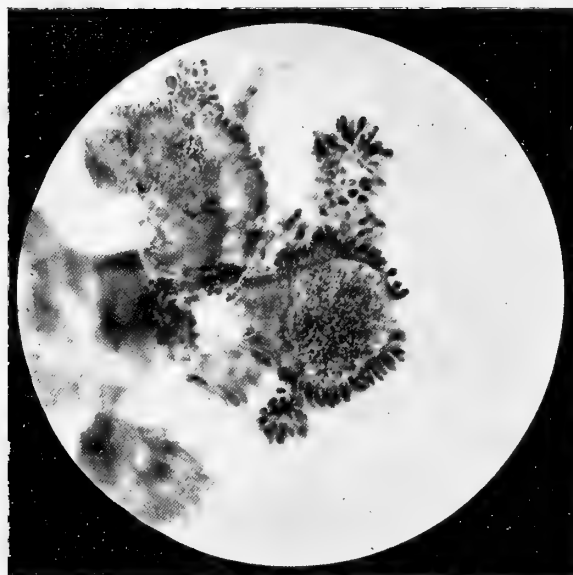


FIG. 2

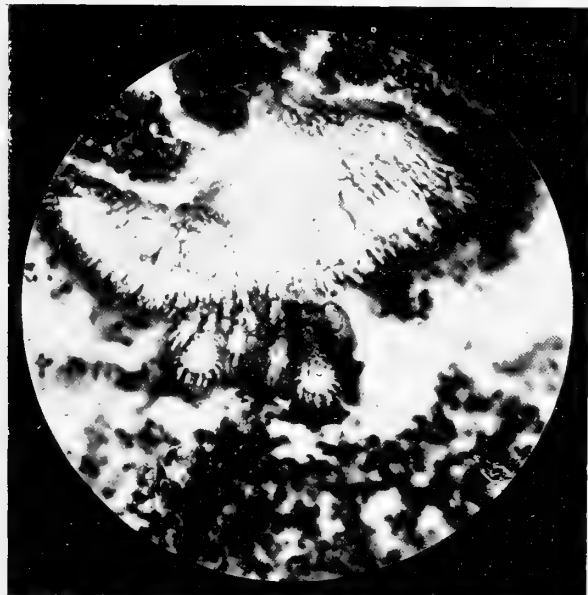


FIG. 3

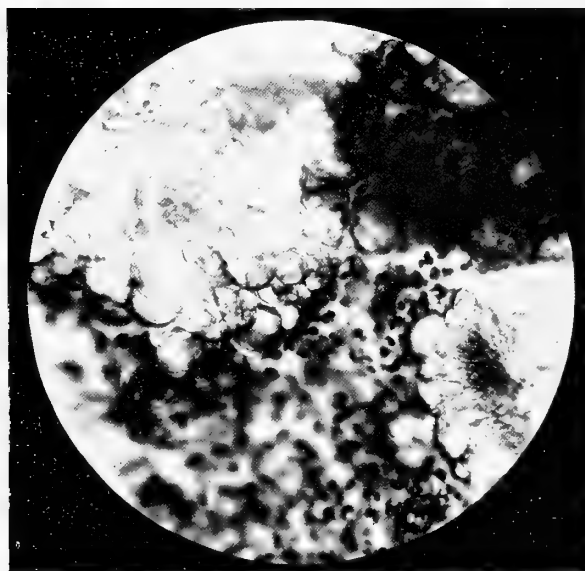


FIG. 4

INOCULATION EXPERIMENTS

Three guinea pigs were inoculated, December 2nd, 1901, subcutaneously in the groin with an emulsion made by crushing the contents of several of the nodules in sterilised water ; about 1 c.c. of the emulsion was inoculated into each guinea pig. At the end of one week the animals were examined, in two no reaction could be felt at the seat of inoculation, but in the third there was a slight swelling. The animals were kept under observation till December 21st. Nos. 1 and 2 still showed no trace of reaction, and in No. 3 the hard swelling had slightly decreased in size ; it was, therefore, determined to kill and examine the guinea pig which showed the reaction. The autopsy revealed a small circumscribed local swelling at the seat of inoculation, and with this exception, all the organs appeared quite normal. A coverslip preparation was made from the swelling and stained and examined, but no hyphae or bacteria were found. The tissue was then hardened and stained, as in the case of the original nodules.

Careful microscopic examination of these sections showed numerous fragments of club-like bodies, and of bits of the ray-like nodules scattered amongst leucocytes and lymphoid cells. There was not a trace, however, of any hyphal structure, or anything approaching the tufts of the true ray fungus. *These experiments show that the fungus-like masses in the nodules, if they really were of the nature of a fungus-like actinomyces, were not alive at the time of inoculation.*

MICROSCOPICAL STRUCTURE OF THE TISSUE.

The pieces of the mammary gland, containing the circumscribed nodules, were hardened in four per cent. solution of formaline and cut in paraffin.

The sections were stained by the following methods :—

1. Haematoxylin.
2. Methylene Blue.
3. Gram's method.
4. Fuchsin and Methylene Blue.
5. Phenol-Thionin.

Fig. 1 is a section under the low power ($1\frac{1}{2}''$ obj.) which shows about one-third of a nodule embedded in the interstitial tissue of the udder, and surrounded by gland tissue. The small violet mass in the nodule represents the mulberry-like mass, stained by GRAM's method. Outside the nodule the mammary tissue appears normal, and is that of the actively secreting gland. Under the high power the nodule is seen to be made up of numerous small round cells, consisting of lymphoid cells, connective tissue cells, and leucocytes, there are no giant cells. The periphery of

the nodule is composed of fibrous tissue separating it from the gland tissue, and there is a nest-like arrangement of the connective tissue around each fungus-like mass, which recalls the appearance of an actinomycotic granuloma. The presence of leucocytes, lymphoid cells, and proliferating connective tissue cells towards the centre, and concentric layers of fibrous tissue at the periphery suggests that the focus is the result of irritation, and that the cause of the irritation is the fungus-like mass.

STRUCTURE OF THE RAY FUNGUS LIKE MASSES

The method of inoculation having failed to furnish any proof of the parasitic nature of the nodules, sections were cut and treated with the above stains, as well as by numerous chemical reagents.

STAINING REACTIONS

Haematoxylin. The Actinomycotic-like masses remained partly unstained and highly refractive, and partly stained red, owing probably to the presence of calcareous salts. The club-like radiate structure can be readily made out. Leucocytes are massed around the ends of the clubs.

Gram's Method. Some of the club-like bodies retain the stain, others remain clear and refractive.

Methylene Blue. The clubs for the most part remain unstained and highly refractive. Where staining does occur the colour is green.

Phenol Thionin. Good differential staining effect is obtained, the fungus-like masses, if they stain at all, assume a green colour.

Fuchsin and Methylene Blue. The fungus-like masses in places stain intensely with the fuchsin.

The staining reactions show that there is little uniformity, the clubs do not invariably stain, and there is no trace of thread-like hyphae. The structure is essentially radiate, but here and there are transparent structureless areas. The material is very refractile, recalling the appearance of a crystalline body. In Figs. 2, 3, and 4 the irregular appearances presented by the mulberry masses is well seen; the clubs vary in size, some are narrow, others greatly thickened at the ends, and some have a distinctly globular configuration, Fig. 4. The clubs may branch, Fig. 3. There is no central core in the club which stains differently from a surrounding capsule; the club as a whole stains or refuses to take the stain. The clubs are for the most part homogeneous, occasionally they are granular, and occasionally there is the appearance of concentric deposits upon them. Thus, as the results of the staining reactions, the masses may be either old degenerate masses of the ray fungus or crystalline formations or concretions.

To try and further determine the point I treated the sections with dilute mineral acids. These caused slight effervescence no doubt from the presence of calcareous material, but they did not materially alter the structure, when the strength of the acid was increased the particles dissolved. Sections treated with dilute caustic potash or sodium hypochlorite, reagents which I had found of the greatest use in removing the organic matter which completely obscured the structure of the Madura fungus, were without effect and in strong solution they caused the particles to dissolve. I next dissected out carefully from the original tissue the fungus-like masses, and tested for fat, leucin, and tyrosin. The reactions for fat and leucin were negative, but I obtained a strong tyrosin reaction by SCHERER'S Test.

There appears to me, therefore, to be little doubt that tyrosin is present in considerable quantity in the crystalline masses, but from the smallness of the material at my disposal I could not say whether they were entirely tyrosin masses.

From the preceding evidence I am inclined to believe that the masses are crystalline, and composed of some substance of the nature of tyrosin, and that they do not represent degenerate forms of the Ray Fungus. Nevertheless, it seems remarkable that a crystalline substance could itself excite an inflammatory reaction. It may be that a pyogenic organism first started an inflammatory reaction in the mamma, and that the deposition of crystalline products was a result of this action. On the other hand, crystals are found in tissues associated with considerable signs of chronic irritation, such as oxalic acid in the kidney, and sodium urate and guanin in connective tissue and cartilage.

If the inflammation producing body was the Ray Fungus, then its distribution in the udder was very peculiar. Every organ in the cow was healthy with the exception that there was a hard calcareous nodule of the size of a pea in the lung, which microscopic examination showed not to be like the abnormal nodules in the udder. The fungus would, therefore, in all probability have gained access to the udder through the teat or skin covering the mamma, but there was no inflammatory extension from either, nor was there a great mass of inflammatory tissue; there were simply small isolated nodules scattered through a portion of the mamma. The supramammary and popliteal glands appeared quite normal.

If, therefore, the case is, as I assume it to be, not one of Actinomyces, it shows, I think, that care should be taken to very clearly prove the presence of fungus cell elements in cases where Actinomyces is reported in an organ like the udder. A number of cases have been recorded of Actinomycosis of the udder. JENSEN¹ describes three cases which were very like acute tubercle. I do not think that the descriptions are sufficiently complete to enable one to judge whether these cases were true Actinomyces or not. In 1886 HERTWIG² described a curious case of Ray

1. Jensen. *Baumgarten's Jahresbericht*, 1893.

2. Hertwig. *Archiv. f. Tierheilkunde* B. 12, 1886.

Fungus discovered by DUNCKER in swine flesh. Looking at the drawings, the appearances certainly rather suggest crystalline formations than fungus growths. They were accompanied by remarkably little inflammatory reaction, and treatment with acid caused the fungus bodies to disappear. In 1901, Mr. PAUL,¹ in a paper upon chronic mastitis, mentions four cases, which on section showed bodies resembling those met with in actinomycosis. As previously mentioned, I regarded them rather as of the nature of degeneration products, and a Committee of the Pathological Society, subsequently appointed to examine the sections, concluded that the striated bodies were due to cell degeneration.

My thanks are due to Dr. Griffith, the Alexander Fellow, for cutting and staining the sections.

1. Paul. Chronic mastitis in its relation to tumour formation. *Pro. Path. Soc.*, Vol. 52, Part I, 1901.

AN ISOLATED CASE OF PLAGUE

AN ISOLATED CASE OF PLAGUE

By A. STANLEY GRIFFITH, M.D. VICT.

ALEXANDER FELLOW IN PATHOLOGY

This case, the leading features of which I shall briefly describe, shows the importance, and, from a public health point of view, the necessity of a systematic examination of all pathological material of doubtful nature derived from hospitals. The case was remarkable in that, although it was the first one of the recent small epidemic in Liverpool to be *definitely proved* to be plague, no connexion could be traced with cases that subsequently occurred, or with those which, by reference to death returns, were strongly suspicious of plague. The positive diagnosis was not made until the end of the tenth day, and this was mainly owing to the fact that the guinea pig inoculated with a piece of the enlarged gland did not die for nearly nine days, and it was felt that the chain of evidence was not complete without this additional link. The early microscopical and cultural appearances also were sufficient only to foster the suspicion of plague, for in the former case the film preparations showed very few organisms, whereas reference to text-books led one to expect that films made from a plague gland would resemble a pure culture, whilst in the latter case the rapid involution of the primary cultures, with the almost complete absence of regular forms at the end of forty-eight hours, prevented the expression of a positive opinion.

The patient was a young man, aged 19, employed in a linseed oil mill. He was admitted to the Medical Wards of the Workhouse Infirmary on September 28, under the charge of Dr. NEVINS.

His illness began on September 26 with headache, vomiting, and 'strangeness in his manner.'

On admission his temperature was 103.2° ; there was a little delirium, photophobia, and irritability; there was also in the left groin a small swelling, which the patient said was due to a kick received whilst playing football. On the second day his temperature rose to 104° , falling the morning after to 99.5° . He continued in much the same condition, with almost constant delirium, until October 2, when it was decided by Dr. ALEXANDER to cut down on the swelling. At the operation no pus was found, only some soft dark-coloured masses, which were removed. Dr. ALEXANDER advised operation because he thought that the swelling in the groin might be due to an inflammatory process set up by the kick, and that the patient's general condition might be relieved by the free drainage of any septic material.

The temperature fell after the operation to 97.4° , but rose again in the evening to 104.2° ; death occurred on the evening of the following day. Some hours before death he complained of acute pain in his throat; the tonsils and glands in the neck were found to be greatly swollen. Immediately after death the tissues of the neck became quite black.

POST-MORTEM

The *post-mortem* was performed by Dr. PEET (R.M.O); the organs were, unfortunately, not preserved for examination. The body showed advanced decomposition, and much *post-mortem* discoloration; the spleen was enlarged and dark, and there were some soft, red, enlarged glands at the back of the pharynx and in the neck. There were no extravasations or ecchymoses, and the organs merely presented changes common to other pyrexial and specific disorders.

MICROSCOPICAL APPEARANCE OF THE GLANDS

At the operation about six to eight glands were removed from the groin, one or two of which were enlarged to the size of a walnut. The glands were soft and dark coloured, and the surrounding interstitial tissue was infiltrated with serum and blood. On section the greater part of a gland seemed to be composed of blood clot; the colour was deep red, varied with a few small greyish necrotic patches, and also numerous small dark haemorrhagic points (engorged blood vessels). There was no evidence of any previous inflammation.

These features, so unlike anything Dr. ALEXANDER was familiar with, suggested to him the possibility that the swelling was a plague bubo. The idea was not then seriously entertained because there was nothing in the youth's history to support the diagnosis: he had lived all his life in Liverpool; he was not brought into contact, either in his employment or in his home, with anything that might ordinarily be supposed to carry infection, and at that time there had been no recorded case of plague in the city. It may be of interest here to remark that no case of plague arose amongst his fellow-workers, his relations, or anyone with whom he had been brought into contact.

MICROSCOPICAL FEATURES

A smear preparation from the cut surface of a gland was made and stained with methylene blue. The film showed leucocytes, red blood corpuscles, a quantity of granular debris, and a few bacilli. The bacilli occurred very infrequently, and appeared to be fairly long and evenly stained; there were, however, two or three which were short and oval, and which showed a distinct tendency to polar staining.

Pieces of the gland were embedded in paraffin and sections cut and stained with the various aniline dyes. The stain, from which the best results were obtained, was a weak solution of carbol-thionin blue, the 'pole staining' being specially well demonstrated by its use.

In sections of the glands many small areas of necrotic softening were seen; the vessels were dilated and engorged with blood, and there was extensive haemorrhagic infiltration into the gland substance and into the interstitial tissue surrounding the gland.

Bacilli were found scattered amongst the cells, sparsely where the gland tissue was not much affected, abundantly in the necrotic areas.

It is not improbable that the first smear preparations did not include the necrotic areas, and this would account for the small number of bacilli in those preparations.

CULTURES FROM THE GLANDS

Agar and serum tubes were smeared and incubated at 38° C. At the end of forty-eight hours, when the tubes were examined, the surface of the medium in each case was seen to be covered with small, circular, prominent, translucent colonies.

Films made from these colonies showed a number of large, irregular, vacuolated and badly stained bacilli, with a very few short oval bacilli, some of which were more deeply stained at the poles than at the centre.

Subcultures were made from individual colonies and pure cultures were obtained. Flasks of broth were then inoculated in the manner recommended by Dr. BALFOUR STEWART in these reports, and incubated at 38° C.

Stalactite formation was observed on the third day.

INOCULATION EXPERIMENTS

Three hours after removal of the glands a guinea pig was inoculated with a small piece of one gland (about a square quarter-inch) beneath the skin of the abdominal wall. On the evening of the eighth day the guinea pig died; the *post-mortem* was made on the following morning.

Post-mortem.—At the site of the inoculation there was a large necrotic abscess. The spleen was enlarged and sown with small white nodules, the condition closely resembling that produced in general tuberculosis (no tubercle bacilli were found). No enlarged glands were seen in any part of the body. In the lungs there were numerous patches of consolidation; the patches varied in size from that of a pin-head to a pea, those near the surface forming distinct elevations beneath the pleura. The centre of the consolidation was greyish-white, whilst around the nodule was a dark, livid, purplish zone.

Films were made and a short oval bacillus showing polar staining was observed in the pus from the local reaction, and in the juice from the spleen and lungs. The preparation from the spleen showed only a few organisms, but that from the lungs resembled a pure culture.

No organisms were detected in films made from the blood.

Pure cultures were obtained from the spleen and from the lungs. The blood was found to be sterile.

A sterile water emulsion of a one day old culture of the organism derived from the lung was inoculated into two rats and one guinea pig. One rat was inoculated in the thigh with a very minute quantity ; the other two animals subcutaneously beneath the skin of the abdomen. The guinea pig died in thirty-six hours. The two rats succumbed at about the same time in a little over two days.

All these animals died of a typical septicaemia, the blood in each case swarming with the characteristic organisms.

With cultures from the blood of these animals 'stalactites' were very rapidly produced in flasks of broth.

The interest of the case lies : (1) in its discovery before the occurrence of other suspicious cases ; (2) in its apparently complete isolation from the subsequent cases ; (3) in the result of the inoculation of the guinea pig, in which, not a septicaemia, but a plague pneumonia developed—this was probably due to the fact that a piece of the gland was used, and not, as in the other animals, a pure culture of the bacillus.

A NEW PATHOGENIC BACILLUS ISOLATED FROM A
CASE DIAGNOSED AS TYPHOID FEVER

WITH A SUMMARY OF FOURTEEN SIMILAR CASES
HITHERTO REPORTED

A NEW PATHOGENIC BACILLUS ISOLATED FROM A CASE DIAGNOSED AS TYPHOID FEVER

WITH A SUMMARY OF FOURTEEN SIMILAR CASES HITHERTO REPORTED

BY

EDWARD H. HUME, B.A. (YALEN.), M.D. (JOHNS HOPKINS)

J. W. GARRETT FELLOW IN PATHOLOGY

'The possibility of other infective agents (colon, paracolon) causing *typhoid-like* symptoms, may explain many "negative reactions in typhoid," and correspondingly enhances the value of the serum-reaction.'

With these words GWYN,¹⁵ in 1898, concluded the report of a remarkable case of fever, clinically like typhoid; and at the same time gave expression to a belief which is gaining ground among investigators of typhoid fever.

It may not be out of place to contrast, at the outset, the wide range of meaning of the term *typhoid-like* when applied to bacilli, with its limitation when used in reference to clinical cases. Of the latter only a comparatively few are on record; while the number of *typhoid-like* bacilli is legion.

I. DEFINITION OF THE TERM 'TYPHOID-LIKE BACILLUS'

GERMANO and MAUREA,¹² in 1893, began their important paper with the statement that by *typhoid-like* bacilli they meant those organisms, motile or not, whose colonies on gelatin plates resembled those of *B. typhosus*. Their paper shows, however, that they must have regarded such similarity as a very superficial criterion, for they pointed out that in order to be really *like B. typhosus*, an organism must not produce gas in sugar media, nor clot milk.

LÖSENER,²⁴ in 1895, summed up mostly clearly the cultural relations which must be fulfilled by a true *B. typhosus*, and his cultural standard remains practically unaltered to-day.

A new criterion was introduced, however, with the discovery of the serum-reaction in 1896, and now no organism can be identified with *B. typhosus*, unless in addition to cultural agreement with a known strain of that bacillus, it is readily agglutinated

by the serum of an animal immunized against typhoid, and that, too, in great dilution. This double requirement has been insisted on by several writers, and in particular by STERN,³⁹ BIBERSTEIN,² JATTA,¹⁹ and WEICHARDT.⁴¹

It would not be reasonable to criticize the use of the expression *typhoid-like*, as applied to bacilli described before 1896, but the organisms described since then may be considered in three groups, as follows :—

1. Bacilli which satisfy only LÖSENER'S²⁴ cultural criterion ; including
 - (a) KISTER'S²¹ bacillus, isolated from water.
 - (b) APPEL'S¹ *Bacillus* Kaiser found in a case of bacteriuria.*
2. Bacilli which satisfy only the agglutinative criterion ; including
 - (a) STERNBERG'S⁴⁰ bacilli, isolated from water.
 - (b) Several members of the *B. enteritidis* group, as described by DURHAM.⁸
3. Bacilli which do not agree with *B. typhosus* in either cultural or agglutinative characteristics ; including
 - (a) SCHOTTMÜLLER'S^{35, 36} bacilli, isolated from blood of patients.
 - (b) KURTH'S²³ bacilli, isolated from blood of patients.

Judged by our present knowledge of *B. typhosus*, the bacilli of the third group can hardly be said to deserve the appellation typhoid-like. This term might well be reserved for organisms which, like those in the first and second groups above, agree with *B. typhosus* in satisfying either the cultural or the agglutinative standard.

II. INFECTIONS CLINICALLY TYPHOID-LIKE, CAUSED BY ORGANISMS OTHER THAN *B. TYPHOSUS*

A record of fourteen such cases has been found in the literature ; for convenience in comparison, the clinical features of these cases have been brought together in the following table

* Houston's (17) four bacilli, isolated from the mud of the River Thames cannot be included here, because
 (a) No mention is made of their action in lactose media.
 (b) They do not alter milk.

TABLE I (A blank space implies absence of record)

No.	Case Reported by :	Age and Sex	Onset	Character of Fever	Rosola (whether present)	Spleen (whether palpable)	Action of Bowels	Diazo-reaction	SERUM-REACTIONS		Duration (in days)	Termination	Complications	Sequelae
									With B. typhosus	With Bacillus isolated				
1	Gwyn ¹⁴ ...	Male (age ?)	Headache ; fever ; weakness	Typhoid-like	+	+	Diarr.	+	o	+		Lysis	3 haemorrhages from bowels on day 30 ; delirium	o
2	Cushing ⁵ ...	Male, 26		ditto	+	+			o	+	70 (?)	Lysis	Relapse ; beginning with epistaxis	Costo-chondral osteomyelitis
3	SCHOTTMÜLLER ¹⁵	Male, 26	Headache ; 3 days prodromal stage, temp. subnormal	ditto	+	+	Constip.		o	+	44	Lysis	Relapse ; bronchitis	o
4	KURTH ²³ (a) Ahl. ...	Male, 29	Headache ; diarrhoea	Remittent ; critical fall	+	+	Diarr.	+	o	+	17	Crisis	o	o
5	(b) Pur. ...	Male, 25	Headache ; vomiting	High fever ; critical fall	+	+	Diarr.	+	o	+	14	Crisis	Bronchitis	o
6	(c) Bg. ...	Female, 30	Diarrhoea	Typhoid-like	+	+	Constip.		o	+	24	Lysis	Bronchitis	o
7	(d) War. ...	Male, 18	Fever ; malaise ; diarrhoea	Almost intermittent	+	o	Diarr.	+	o	+	17	Lysis	Transient angina ; transient gastritis during convalescence	o
8	(e) Kru. ...	Female, 23	Headache ; lassitude	Intermittent	doubtful	o	Constip.	o	o	+	27	Lysis	o	o
9	(a) Krenzin	Male, 60	Headache ; lassitude	Typhoid-like	o	Splenic dulness increased	Diarr.		o	+	32	Lysis	Bronchitis ; arthritis during convalescence	o
10	(b) Köcher...	Male, 18	Headache	ditto	+	o	Constip.		o	+	25	Lysis	Bronchitis ; delirium	o
11	(c) Thot ...	Male, 19	Headache ; lassitude ; anorexia	ditto	+	+	Constip.		o	+	18	Lysis	o	o
12	(d) Seemann	Male, 15	Rigor ; diarrhoea ; lassitude ; malaise	ditto	+	o	Diarr. + Constip.	+	o	+	34	Lysis	Ulcer on tonsil ; bronchitis	o
13	(e) Dr. K. ...	Male, 25	Headache ; malaise ; somnolence	Very mild	o	Splenic dulness increased	Constip.		o	+	16	Lysis	o	o
14	(f) Müller ...	Male, 46	Headache ; lassitude	Typhoid-like	+	+	Constip.		o	+	29	Lysis	Bronchitis	o

SUMMARY OF THE CLINICAL FEATURES

Sex of patients : Male, 12 ; female, 2

Average age : (13 cases reported), 27·69 years.

Onset and Course : Typhoid-like, 10 ; atypical, 4.

Roseola : Present in 11, absent in 2, doubtful in 1.

Spleen : Palpable in 8, not felt in 6.

Character of stools : Diarrhoea in 6, constipation in 8, not reported in 1.

Average duration : (13 cases reported), 28 days.

Termination : By lysis, 12 ; by crisis, 2.

Relapse : In 2 cases.

Fatal result : None.

Complications :—

Haemorrhage from bowels	.	1
Haemorrhage from nose	.	1
Delirium	.	2
Arthritis	.	1
Tonsillar ulcer	.	1
Bronchitis	.	7

Sequelae : Only serious in No. 2 (costo-chondral osteomyelitis).

Four cases are set down as atypical ; two of KURTH's,²³ because of a critical fall in temperature ; two others of the same writer's, because of their remarkably intermittent temperature. In fact, KURTH regarded his case 'Kru' as one of gastritis until the patient's serum gave a positive reaction with the bacillus isolated from case 'Ahl,' whose illness ran a 'typically' typhoid course.

The fact of recovery in every case is noteworthy ; especially so in two of SCHOTTMÜLLER's³⁶ cases, for one of them was an alcoholic, and the other a man of sixty.

The record as to the character of the stools is much like that of a series of true typhoid cases, but markedly different from that of cases of meat-poisoning caused by bacilli of the same group (*B. enteritidis* group). Severe diarrhoea and collapse are features of the latter. It is a remarkable fact that a group of organisms should produce on the one hand, in the cases of meat-poisoning, such a marked general enteritis and toxæmia, with an incubation period of but a few hours, and an illness of but a few days ; and on the other hand, as in the present series, a slow infection of typhoid-like course.

The bacteriological findings in the fourteen cases are recorded in Table II.

No.	Case	CHARACTERISTICS					Indol	Relation to Oxygen	Pathogenicity	
		Alkal-red agar	Sulph-indigotate of Soda Solution	Litmus Whey	SUGAR MEDIA					
					Glucose	Lactose				Sacchar-ose
1	GWYN ¹⁴ .. 'Par		Gas	o	o *	trace *	...	
2	CUSHING .. Baci		Gas	o	o	trace	Fatal to mice	
3	SCHOTT ¹⁵ ; de- Bariza- n ;		Gas ; change to yellow	Acid, 1.6 per cent.	Gas	o	...	
4	KURTH ² science (a)	Gas	o	} 1/10 loop fatal to small guinea- pigs ; 1 loop no fatal to large guinea- pigs	
5	(b) —		—	—	—	—	—	—		
6	(c)	Gas	o		
7	(d) —		—	—	—	—	—	—		
8	(e) —		—	—	—	—	—	—		
9	SCHOTT ¹ (a)								All facultative anaerobes	
10	(b) ; de- ariza- n ;		Gas ; change to yellow	Alkaline, 1.8 to 2.8 per cent.	Gas	o		...
11	(c) science									
12	(d)									
13	(e) —		—	—	—	—	—	—		
14	(f) ditto		ditto	Acid, 1.4 per cent.	Gas	o	...	
	B. enteroto		ditto	Alkaline	Gas	o	o	o or trace	Marked	

TABLE II (... implies absence of record.)

No.	Case reported by	ISOLATION OF BACILLI		SERUM-REACTION OF PATIENTS		MORPHOLOGY				CULTURAL CHARACTERISTICS											Indol	Relation to Oxygen	Pathogenicity
		Source	Day of Illness	With B. typhosus	With Bacillus isolated	Form	Motility	STAINING		GELATIN		Potato	Milk	Neutral-red Agar	Sulph indigotate of Soda Solution	Litmus Whey	SUGAR MEDIA						
								With Aniline Dyes	By Gram	Plates	Liquefaction						Glucose	Lactose	Saccharose				
1	GWYN ¹⁴ 'Paracolon'	Blood	26	o	+, 1: 200	Like B. typhosus	+	+	o	Like B. typhosus	o	Like B. typhosus	Acid, then alkaline; no clot	Gas	o	o*	trace*	All facultative anaerobes	...	
2	CUSHING ⁵ Bacillus 'O'	Sinus of costochondral osteomyelitis	9 months after supposed typhoid	o	+, 1: 800	ditto	+	+	o	ditto	o	ditto	ditto	Gas	o	o	trace		Fatal to mice	
3	SCHOTTMÜLLER ³⁵ Barg.	Blood	4	o	+, 1: 100	Short rods: few long forms	+	+	o	ditto	o	ditto	Peptonized; alkaline; no clot	Gas; decolourization; fluorescence	Gas; change to yellow	Acid, 1·6 per cent.	Gas	o		...	
4	KURTH ²³ (a) Ahl.	Faeces	23	o	+, 1: 8000	Like B. typhosus	+	+	o	Round, well-defined colonies	o	...	After many weeks, clot formed	Gas	o		1st loop fatal to small guinea-pigs; 1 loop not fatal to large guinea-pigs	
5	" (b) Pur.	None isolated	—	o	+, 1: 500 (With bacillus from Ahl.)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
6	" (c) Bg.	Urine	45	o	+(Bacillus Bg. with serum Ahl.)	Like B. typhosus	+	+	o	ditto	o	—	ditto	Gas	o			
7	" (d) War.	None isolated	—	o	+, 1: 500 (With bacillus from Ahl.)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
8	" (e) Kru.	None isolated	—	o	+, 1: 250 (With bacillus from Ahl.)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	
9	SCHOTTMÜLLER ¹⁶ (a) Krenzin	Blood	15	o	+, 1: 100	Short rods: few long forms	+	+	o	Fair-sized, yellow colonies; not sulcate	o	Greyish-brown, fairly thick growth	Peptonized; alkaline; no clot	Gas; decolourization; fluorescence	Gas; change to yellow	Alkaline, 1·8 to 2·8 per cent.	Gas	o		...	
10	" (b) Köcher	Blood	4	o	+, 1: 10,000																		
11	" (c) Thot	Blood	8	o	+, 1: 10,000																		
12	" (d) Seemann	Blood	21	o	+, 1: 10,000																		
13	" (e) Dr. K.	None isolated	—	o	+, 1: 100 (With bacilli from cases 9, 10, 11, 12)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		...	
14	" (f) Müller	Blood	14	o	+, 1: 100	ditto	+	+	o	Small, transparent, irregular colonies	o	Moist, scarcely visible growth	ditto	ditto	ditto	Acid, 1·4 per cent.	Gas	o		...	
B. enteritidis group						ditto	+	+	o	Small, rounded, transparent colonies	o	Usually like B. typhosus	Acid, then alkaline; no clot	ditto	ditto	Alkaline	Gas	o	o	o or trace	Marked		

SUMMARY OF THE BACTERIOLOGICAL FEATURES

Cases in which bacilli were isolated, 10.

From blood	.	.	.	7
„ urine	.	.	.	1
„ faeces	.	.	.	1
„ costochondral abscess	.	.	.	1
None isolated in four cases.				

Time of isolation—

During first week of illness	.	.	2
„ second „ „	.	.	2
„ third „ „	.	.	2
„ fourth „ „	.	.	2
„ seventh „ „	.	.	1
Nine months after illness	.	.	1

Serum-reaction of patients—

Negative with <i>B. typhosus</i>	.	.	14
Positive with bacillus isolated from same patient	.	.	9
Positive with bacillus isolated from another patient	.	.	4

IDENTIFICATION OF THE BACILLI ISOLATED

GILBERT,¹³ in 1895, pointed out that there were bacteria allied to *B. coli*, but with greater pathogenicity. He called these 'Para Coli' and divided them into five groups. Most of the bacilli reported in the above table belong, evidently, to his third group, consisting of organisms not fermenting lactose.

A clearer classification was made in 1898 by DURHAM,⁸ who rearranged the so-called 'typhoid-colon' group into three groups, readily differentiable by means of their action in various sugar media. His classification is as follows :—

- I. *B. typhosus* and its allies; produce acid but no gas bubbles in glucose; do not attack lactose.
- II. *B. enteritidis* and its allies; produce acid and gas in glucose; do not attack lactose.
- III. *B. coli* and its allies; produce acid and gas in both glucose and lactose.

By comparison with the cultural features of the *B. enteritidis* group included in Table II, it will be seen that the bacilli are, so far as can be determined from the description, in nearly every case, members of that group. The bacillus described by KURTH²³ may be an exception, because of the terminal clotting in milk. It is greatly to be regretted that so many of the reports fail to give a record of the action of the bacillus described in lactose media. The necessity of recording this action as a means of differential diagnosis was emphasized as early as 1885, by BUCHNER,³ and although it has been repeatedly referred to by other writers, the persistent failure to report on it is a source of difficulty to any one attempting to classify a given bacillus.

The bacilli isolated by GWYN¹⁴ and CUSHING⁵ have been shown by these writers to belong to the *B. enteritidis* group. In this group are also included by DURHAM⁸—

- (a) Various bacilli isolated in epidemics of meat-poisoning, both on the Continent and in England ;
- (b) The so-called gas-producing typhoid bacilli of various observers ;
- (c) The bacilli isolated in certain cases of septicaemic typhoid fever.

The bacilli isolated by SCHOTTMÜLLER,^{35, 36} in six of his cases, clearly belong here too. For although the important lactose reaction is not recorded, nevertheless, the milk reaction is typical of the *B. enteritidis* group ; as has been shown by CUSHING,⁵ who worked with several members of the group and found that they all turned milk alkaline after a period of transient acidity.

It is more difficult to place the bacillus isolated by KURTH.²³ The terminal clotting of milk suggests that lactose would have been fermented, if tried ; if so, the organism would most certainly be a form of *B. coli*. So few, however, of the *B. enteritidis* group have been studied in milk over protracted periods of time that it is impossible to say definitely what change might occur in some of the members.* KURTH's bacillus ('bacillus bremensis febris gastricae') evidently belongs, even though allied to *B. enteritidis*, to Group F, Order I, Division II, of DURHAM's⁹ elaborate classification, published in 1901. He speaks of this group as having colon-like morphology and motility, and as being 'Dextroso-non-lactoso-fractors' ; milk is slightly clotted by them eventually.

It is worthy of special note that SCHOTTMÜLLER should have been so abundantly successful in isolating the aetiological bacillus from the blood of his cases. He reports that in thus examining, in a routine way, the cases of typhoid fever admitted to the Allgemeines Krankenhaus in Hamburg, St. Georg, *B. typhosus* was isolated from the blood in eighty per cent. of the cases in 1899, and in eighty-four per cent. in 1900. It was during the course of such routine examinations that he isolated the bacilli described, in one case as early as the fourth day of the disease.

* Thus MacConkey and Hill (27) in their elaborate tables recently published, record the cultural relations of six members of the *B. enteritidis* group (*i.e.*, *B. enteritidis*, *B. paracolon*, *B. cholerae suum*, *B. icteroides*, *B. psittacosis*, *B. of epidemic jaundice*), and report that all of them acidify milk in forty-eight hours. Although their statement is literally true—that for forty-eight hours cultures of this group in milk are acid—still it is a recognized fact that this acidity is only transient, and that one of the distinctive features of the *B. enteritidis* group is that it turns milk alkaline after forty-eight hours. The writer has repeatedly tested the six cultures used by MacConkey and Hill, and every one of them has turned milk strongly alkaline after forty-eight hours ; this alkalinity persists after many weeks of continuous incubation at 37°C.

III. REPORT OF AN ADDITIONAL CASE

A case recently admitted to the Liverpool Royal Infirmary, as one of typhoid fever, but which gave a negative serum reaction with *B. typhosus*, affords the basis for the present paper. The clinical history is reported by kind permission of Dr. Caton.

T.R., Male, age twenty-nine, bricklayer, admitted to the Royal Infirmary on September 24, 1901, complaining of 'typhoid.'

Family history and personal history : unimportant.

Present illness :—

September 15. Seized with headache, chiefly occipital ; felt sick.

„ 18. Headache became much worse, and there was pain in the back and legs. Went to bed thinking he had influenza.

„ 19. Went for a short walk, but had to go back to bed on returning home. Had several severe attacks of vomiting and diarrhoea.

„ 21. Headache ceased.

On admission, September 24 : Patient is well developed ; lies in dorsal decubitus ; clear mentally ; cheeks flushed ; skin is warm and moist.

Temperature : 101.4° F. (38.5° C.) No jaundice.

Tongue : coated with white fur.

Appetite : poor ; is very thirsty ; no diarrhoea or vomiting within first few hours.

Circulatory System : pulse 80 ; regular ; low tension ; large volume ; artery wall normal. Heart : not enlarged. Faint systolic murmur at apex ; no other murmurs.

Respiratory System : chest rather emphysematous ; no bronchitis ; lungs clear.

Nervous System : no headache ; sleeps poorly ; reflexes sluggish ; mind clear.

Urinary System : micturition normal ; urine normal ; no albumin.

Roseola : there are numerous rose spots on chest and abdomen ; these disappear on pressure.

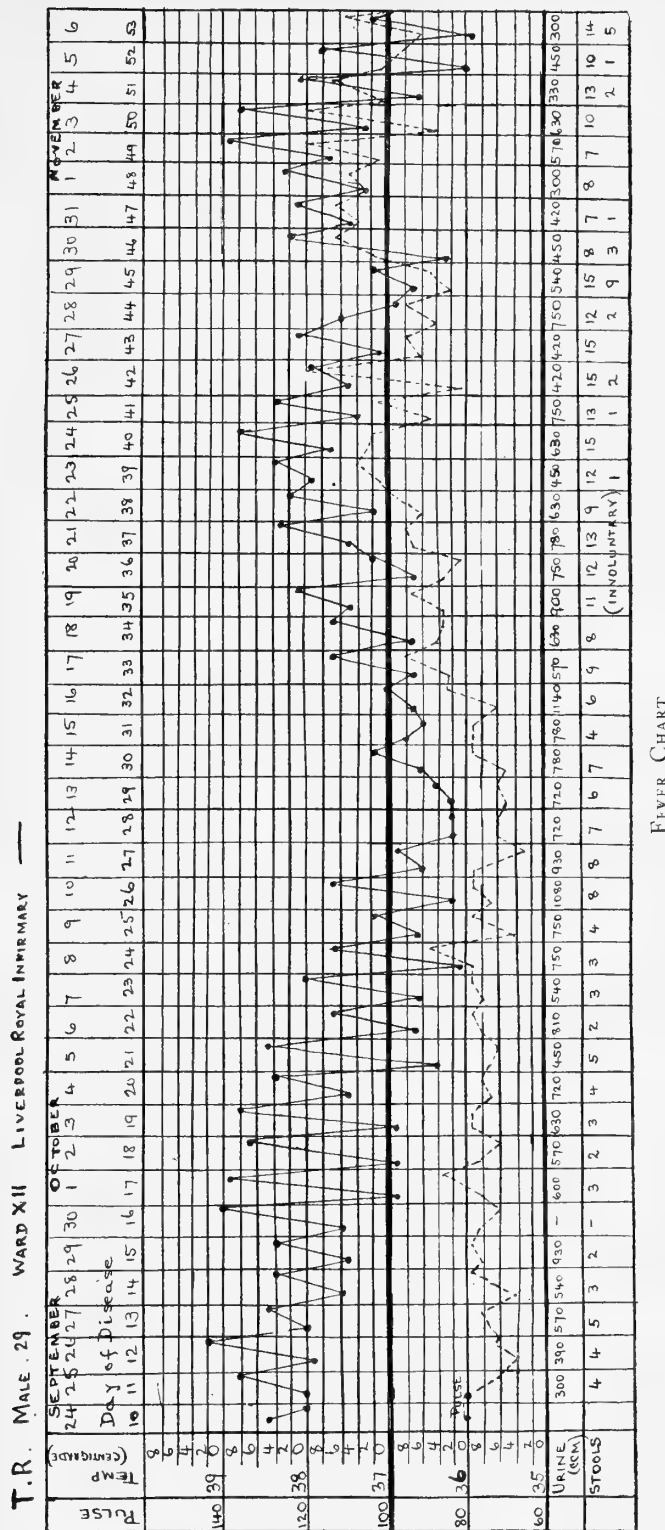
Spleen : palpable on deep inspiration.

Abdomen not distended.

September 30. Evening temperature still above 102° (39° C.) ; slight cough ; considerable muco-purulent expectoration ; roseola still present.

- October 7. Temperature coming down ; tongue cleaner.
- „ 8. Some blood in stools.
- „ 14. Blood still present in each movement ; considerable diarrhoea.
- „ 17. Temperature rising again.
- „ 24. Diarrhoea severe ; no blood in stools ; one involuntary movement during night ; temperature rose to 101.8 (38.8°) at 10 p.m.
- „ 28. Urine contains albumin, but no blood ; sp. gr. 1020.
- „ 29. Considerable haemorrhage from bowels ; ergotin 0.0006 grm.
- „ 31. Haemorrhage ceased.
- November 6. During past four days the temperature has reached 102° (39° C.) each evening.
- „ 7. Temperature normal ; diarrhoea still continues.
- „ 18. Patient feels stronger ; diarrhoea less ; no involuntary motions.
- „ 28. Cultures made from stools.
- December 6. Urination painful ; 540 ccm. of alkaline, purulent urine withdrawn by catheterization.
- „ 7. 240 ccm. of urine drawn ; temperature rose to 101.2° (38.4° C.)
- „ 9. Cultures made from urine ; urine clearer and acid.
- „ 15. Patient was up for one hour.
- „ 17. Cystitis recurring.
- „ 21. Urine clearer, but still alkaline.
- „ 23. Discharged ; quite convalescent but very weak ; appetite good ; diarrhoea practically ceased.

A chart showing the temperature and pulse curves is appended.



FEVER CHART

The points of interest in the case are :—

- (a) The presence of typical typhoid onset and symptoms ; roseola, enlarged spleen, and a fairly typical temperature curve.
- (b) The profuse and long continued diarrhoea, for a time with bloody stools, and for a week or more with involuntary movements. The diarrhoea continued for more than a month after the temperature became normal.
- (c) A well-marked relapse.
- (d) The occurrence of a cystitis during the convalescence.

ISOLATION OF BACILLUS 'L' FROM FAECES AND URINE

On November 28, three weeks after the temperature became normal, cultures were first made from the stools, a small quantity being collected in a sterile jar. Plates were made on MACCONKEY'S²⁶ taurocholate agar, and loopfuls of the faeces were inoculated into each of several tubes made according to a modification of GABRITSCHESKY'S¹¹ pattern. The latter are designed to facilitate isolation from stools, of organisms like *B. typhosus*, whose motility is usually greater than that of *B. coli*. Within twenty-four hours pure cultures were obtained from the distal end of the tubes, of a motile bacillus in form like *B. typhosus*. Sub-cultures on various media soon indicated that the organism was evidently a member of the *B. enteritidis* group. A few days after the first isolation of the bacillus, the patient developed a cystitis. Cultures were made as before, from a portion of catheterized urine ; on the plates were found, in addition to organisms identified as *B. coli*, a large number of colonies of a bacillus soon proved to be the same as that isolated from the stools.

A full comparative study of the organism thus isolated from both faeces and urine was undertaken, the results of which are appended. The bacillus will be spoken of throughout this paper as *Bacillus* 'L.'

AGGLUTINATION TESTS WITH PATIENT'S SERUM

The patient's serum-reaction was, unfortunately, not tried during the acute stage of his illness ; and yet the results recorded below were so positive as to lead one to the conclusion that the typhoid-like illness was due to an infection, not with *B. typhosus*, but with *Bacillus* 'L.' The serum was tested on November 28, seventy-five days after the onset of the symptoms and twenty-one days after the temperature became normal. The results of the test, made with six available cultures of the *B. enteritidis* group, as well as with cultures of *B. typhosus* and *B. coli* are recorded in Table III ; while in Table IV is shown the action of the sera of two typhoid patients and two normal persons on some of the bacilli recorded in Table III.

TABLE III. SERUM-REACTIONS OF T.R.

N.B.—All serum-reactions made with emulsions in 1·0 per cent. NaCl solution, of sixteen-hour agar cultures. Emulsions filtered before use. Dilutions made with a graduated capillary pipette. All determinations microscopic.

Dilution and Time-limit	Bacillus 'L' (from stools)	Bacillus 'L' (from urine)	B. typhosus (B.T.A.)	B. typhosus (A.D.)	B. enteritidis (Gärtner)	B. cholerae suum (Salmon & Smith)	B. icteroides	B. psittacosis (Nocard)	B. of epidemic jaundice	B. paracolon (Le Sage)	B. coli (Escherich)
1 : 10— $\frac{1}{2}$ hr.	+	+	o	o	+	—	—	—	—	+	+
1 : 50—1 hr.	+	+	o	o	o	o	o	o	o	o	tr.
1 : 100—2 hrs.	+	+	o	o	o	o	o	o	o	o	tr.
1 : 200—2 hrs.	+	+	o	o	o	—	—	—	—	—	o

+ Positive. tr. Trace. o No clumping. — Not tried.

TABLE IV

Source of Serum	Dilution and Time-limit	Bacillus 'L'	B. typhosus (B.T.A.)	B. enteritidis	B. Coli (Escherich)
Typhoid Patient (443)	1: 10— $\frac{1}{2}$ hour	+	—	*	+
	1: 30— $\frac{1}{2}$ hour	tr	+	*	+
	1: 50—1 hour	tr	+	+	+
	1: 100—2 hours	tr	+	*	+
	1: 200—2 hours	o	+	tr	tr
Typhoid Patient (445)	1: 10— $\frac{1}{2}$ hour	+	+	+	+
	1: 30— $\frac{1}{2}$ hour	o	+	+	+
	1: 50—1 hour	o	+	+	+
	1: 100—2 hours	o	+	tr	+
	1: 200—2 hours	—	+	—	—
Normal Person (E.N.C.)	1: 10— $\frac{1}{2}$ hour	+	+	—	+
	1: 50—1 hour	o	o	—	+
Normal Person (E.H.H.)	1: 10— $\frac{1}{2}$ hour	+	+	—	+
	1: 50—1 hour	o	o	—	+

+, Positive. *, Partial. tr, Trace. o, No clumping. —, Not tried.

It is to be noted that in dilution 1 : 50, *B. enteritidis* was clumped by the sera from the two known cases of typhoid.* *Bacillus* 'L' was also clumped, although to a less extent, by one of the typhoid sera. The interaction of these allied bacilli and their sera was more fully studied by the use of immunized animals; reference to these studies will be made later.

COMPARATIVE BACTERIOLOGICAL STUDY OF *BACILLUS* 'L'

Inoculations were made upon all media with the following organisms :—

- A. *B. typhosus* group.
 - 1. *B. typhosus* (A.D.) ; turns milk alkaline after a week.
 - 2. *B. typhosus* (L.C.) ; milk remains acid after many weeks.
 - 3. *B. typhosus* (B.T.A.) ; milk remains acid after many weeks.
- B. *B. enteritidis* group.
 - 1. *B. enteritidis* (GÄRTNER).
 - 2. *B. paracolon* (LE SAGE).
- C. *B. coli* group.
 - 1. *B. coli* (ESCHERICH) ; does not ferment saccharose.
 - 2. *B. coli* ('Communiør'†) ; ferments saccharose.

The results were compared with simultaneous inoculations of two cultures of *Bacillus* 'L,' one isolated from the faeces, the other from the urine of the patient. With minor variations, the results of the inoculations were so constant within each of the above groups—A, B, and C—that for the sake of conciseness, *Bacillus* 'L' will be compared with the three groups as units rather than with individual bacilli. (See Table V as appended.)

NOTES ON THE METHODS OF STUDY

Morphology. The only observed difference between *Bacillus* 'L' and *B. typhosus* lay in the regularly observed polar staining of the former. This was best seen when young agar cultures were lightly stained with Carbol fuchsin; it was not so well brought out by Methylene blue, but was clearly made out by the use of ZETTNOW's⁴ silver method.

Flagella. The method of staining found most satisfactory was the one devised by Dr. ZETTNOW⁴; the mordant consisting of antimony tartrate and tannic acid; the stain, of a solution of silver sulphate in ethyl-amine (30 per cent.).

Potato. As has been often pointed out, this method of differentiation is very unreliable. CUSHING⁵ shows that all depends upon the initial reaction of the potato.

* This confirms the opinion expressed by Durham (8), *i.e.*, that in dilutions below 1 : 100, *B. enteritidis* may often react as well as *B. typhosus* with known typhoid sera. To account for this reaction he has since then formulated a theory (9) which certainly helps to explain one of the intricate phenomena of agglutination.

† The expression *B. coli* 'communiør' is borrowed from Durham (9).

Milk. Although, as DURHAM⁹ points out, this medium may not be wholly satisfactory, nevertheless it does form a ready means of differentiating the groups of bacilli under consideration. LUBARSCH's²⁵ statement that *B. enteritidis*-clots milk has been shown by DURHAM,⁸ FLEXNER,¹⁰ and others to be erroneous. CUSHING's⁵ careful study demonstrates the uniformity of alkali production in milk by all the members of the *B. enteritidis* group to which he had access. It was by means of milk cultures that *Bacillus* 'L' was shown not to be a typical member of the *B. enteritidis* group, for while the latter all turned milk alkaline after two days, *Bacillus* 'L' continued to acidify it. Thus :—

Acid formed after one week at 37° C. = 2.25 per cent. normal KOH.

„ „ „ four weeks „ = 3.75 „ „ „

Taurocholate lactose agar. This very useful differential medium synthesized by MACCONKEY²⁶ brought out a characteristic difference between the *B. coli* group and the other two groups studied, *i.e.*, in the formation of a definite haze or halo about the separate colonies of the former. As shown by MACCONKEY, this is due to the fact that bile salts are precipitated by the acid formed from lactose, *B. coli* alone (within these three groups) having the power to ferment this sugar.

Neutral-red media. As pointed out by ROTHBERGER,⁵¹ SCHEFFLER,³⁴ and others, *B. coli* rapidly decolourizes glucose agar containing Neutral-red, while *B. typhosus* produces no change. WOLFF,⁴² and later, RAMBOUSEK,³¹ have claimed that the decolourization is a process of reduction, the addition of two atoms of nascent H forming a 'leuco-product.' It seems likely, however, that other agents as well must be able to decolourize Neutral-red, for in agar containing lactose, *B. enteritidis* and *Bacillus* 'L' (organisms that do not ferment lactose) the characteristic decolourization and fluorescence were observed (see Table VI). If this were due to the presence of a small amount of glucose, either originally present in the agar or formed by overheating of the lactose, gas bubbles should have been seen. Possibly the process is similar to the regularly observed decolourization of litmus in a litmus-lactose-peptone solution by *B. typhosus* (which does not ferment lactose).

MAKGILL²⁸ and SAVAGE,³³ after using the medium for routine water examination, came to the conclusion that if the characteristic reaction were not obtained, *B. coli* was probably absent. HUNTER¹⁸ noted that *B. enteritidis* reacted like *B. coli* in Neutral-red, and, therefore, came to the conclusion that *B. enteritidis* was a modified form of *B. coli*, a view expressed also in LEHMANN and NEUMANN's *Atlas of Bacteriology*. The assumption seems unwarranted, for the following reasons :—

1. The fundamental requirements of a true *B. coli*, as laid down by GILBERT¹³ in 1895, are, that in addition to its motility and production of indol, (a) it shall ferment lactose; (b) it shall clot milk. No member of the *B. enteritidis* group satisfies these two conditions.

	IF
No.	C
	ek
1	Gall l; ci es lin
2	Gas les
3	Br ot
4	G

TABLE V

MORPHOLOGY										CULTURAL CHARACTERISTICS																			
Group	Form	Motility	Flagella	STAINING		GELATINE CULTURES: 48 Hours: 20° C.				GLYCERINE AGAR	BLOOD SERUM	BOUILLON	POTATO (parallel streak with B. typhosus)	LITMUS MILK: 37° C.			TAUROCHOLATE-LACTOSE AGAR	NEUTRAL-RED GLUCOSE AGAR	LACTOSE LITMUS AGAR	NEUTRAL LITMUS WHEY	SUGAR MEDIA: 48 hours: 42° C.				GLYCERINE-PEPTONE-WATER 6 days: 37° C.	Intol	H ₂ S	Relation to Oxygen	
				With aniline dyes	By Gram	Plates	Streak	Stab	Liquefaction	48 hours: 37° C.	48 hours: 37° C.	24 hours: 37° C.	24 hours: 37° C.	2 days	4 days	2 weeks	4 weeks	48 hours: 42° C.	48 hours: 37° C.	48 hours: 37° C.	5 days: 37° C.	Litmus Glucose	Litmus Lactose	Litmus Saccharose	Litmus Mannite				
1. <i>Col. typhosus</i>	Short rods, 1.0—3.0 μ , by 0.6—0.8 μ ; long forms in old cultures; no spores	Active	8-14	Stains well	o	Surface colonies: small, translucent, greyish-white, irregularly rounded, sulcate Deep: yellowish, round or whetstone-shaped	Flat, translucent, whitish, shiny growth; not spreading	Delicate, greyish growth; many discrete colonies	o	Flat, greyish, translucent growth; many small, discrete colonies	Delicate, greyish-white growth; very flat; many discrete colonies	Uniformly turbid; pellicle rare	Usually leucate, moist, scarcely visible growth	Acid: no clot	Acid: no clot	Usually acid; Some cultures alkaline	Usually acid	Surface colonies: Small, semi-transparent, greyish-white; Deep: more opaque; no haze	No change	Blue colonies	Acid	Acid: no gas	No fermentation; decolourization	Nil	Acid: no gas	Acid	o	+	All facultative anaerobes
2. <i>B. typhosus</i>	Like B. typhosus; long forms rare	Active	8-10	Often irregular; poles or centres deeply stained	o	Like B. typhosus, but granular, not sulcate	Like B. typhosus, but more rapid	Like B. typhosus	o	More luxuriant than B. typhosus	Many large, white, coalescing colonies	Uniformly turbid; pellicle regularly	Greyish-white, shiny growth; later, spreading	Transient acidity: no clot	Alkaline	Increasingly alkaline; opalescent; no clot	Like B. typhosus; no haze	Gas: decolourization; fluorescence	Blue colonies	Alkaline after transient acidity	Acid; gas	Like B. typhosus	No fermentation; decolourization	Acid: gas	Neutral or faintly acid	o	+		
3. <i>B. enteritidis</i>	Like B. typhosus; long forms very rare	Less active than B. typhosus	8-10	Stains well; usually polar staining	o	Like B. enteritidis	Like B. enteritidis	More luxuriant than B. typhosus	o	Like B. enteritidis	Like B. enteritidis	Like B. enteritidis	More luxuriant than B. enteritidis	Acid: no clot	Acid; no clot	Increasingly acid; no clot after six weeks	Like B. typhosus; no haze	Like B. enteritidis	Blue colonies	Acid	Acid: gas	Like B. typhosus	Like B. enteritidis	Acid: gas	Neutral or faintly acid	+	+		
4. <i>Col. bacillus 'L'</i>	Usually short rods; long forms frequent	Usually sluggish	4-8	Stains well	o	Surface colonies: opaque, yellowish, round, granular Deep: yellow, whetstone-shaped	Raised, yellowish, opaque, shiny, spreading growth	Like bacillus 'L'	o	Profuse spreading, greyish growth	Profuse growth; no discrete colonies	Very turbid, pellicle variable	Profuse, yellow, raised, spreading growth; later, brown	Acid: clot	Surface colonies: Large, opaque, yellowish, spreading; many with orange centres; haze	Like B. enteritidis	Red colonies	Acid	Acid: gas	Acid: gas	Acid: gas (ESCHERICH'S B. coli does not ferment saccharose)	Acid: gas	Acid	+	+	

2. The statement of LEHMANN and NEUMANN that *B. enteritidis* clots milk is based entirely upon LUBARSCH'S²⁵ paper ; no other observer has noted a similar result. On the contrary, it is an accepted characteristic of the entire *B. enteritidis* group, that they do not clot milk.
3. As shown by DURHAM,⁹ the members of the *B. enteritidis* group are not agglutinated by the serum of animals immunized with *B. coli*.

The present study has led to the conclusion that *because the Neutral-red reaction occurs, bacilli capable of causing typhoid-like symptoms cannot be excluded*, for every member of the *B. enteritidis* group has been found to give the characteristic reaction. In order to test the matter thoroughly, two series of media were prepared ; one containing glucose, the other lactose, in view of the fact that the latter sugar is fermented by *B. coli*, and not by the other groups. To each medium was added one per cent. of a saturated watery solution of Neutral-red. Three strains of *B. typhosus*, nine of the *B. enteritidis* group, and three of *B. coli*, as well as a culture of *B. faecalis alkali-genes*, were used for comparison. The results are shown in the following table :—

TABLE VI

Shewing the comparative action of five groups in Sugar media containing 1 per cent. saturated aqueous solution of Neutral-red.

	0·5 PER CENT. GLUCOSE MEDIA 48 HOURS : 37° C.			0·5 PER CENT. LACTOSE MEDIA 48 HOURS : 37° C.	
	Agar Shake	Bouillon	Peptone Water	Agar Shake	Peptone Water
<i>B. typhosus</i>	o	T A	Slight T A	o	T Alk.
<i>B. enteritidis</i>	G D F	T A	T A	D F	T Alk.
<i>Bacillus 'L'</i>	G D F	T A	T A	D F	T Alk.
<i>B. coli</i>	G D F	T A	T A	G D F	T A
<i>B. faecalis alkali-genes</i> ...	o	T Alk	o	o	T Alk.

o, no change. G, gas. D, decolourization. F, fluorescence. T, turbidity. A, acid. Alk, alkaline.

The characteristic decolourization was not seen in fluid glucose media. It was strikingly brought out, however, that Neutral-red lactose peptone water would differentiate *B. coli* from the other groups far more sharply than Neutral-red glucose media. In such a medium the cultures of *B. coli* changed the Neutral-red colour to cherry-red. (A similar change of colour was observed on the addition of one drop of acid, mineral or organic). For purposes of comparative study this medium proved fully as satisfactory as PETRUSCHKY'S³⁰ Neutral litmus whey; its constitution is certainly more constant than that of the latter medium. Merely as a qualitative medium, Neutral-red may be more useful than litmus in the study of these particular groups, because of the frequent decolourization of the latter; but for rapid differential work Neutral-red can hardly be considered a valuable adjunct, especially as used by those who have recommended it hitherto; glucose agar will differentiate the groups as well without the addition of Neutral-red as with it.

Lactose litmus agar. First synthesized by WURTZ,⁴³ and modified later by KASHIDA,²⁰ this medium has been very recently brought into prominence by VON DRIGALSKI and CONRADI,⁶ who have succeeded by its use in isolating *B. typhosus* from the stools of each of fifty typhoid patients examined, as well as from the stools of several individuals, attendants in typhoid wards, who had shown no symptoms of illness.

Neutral litmus whey. At DURHAM'S⁹ suggestion the whey was neutralized, not with HCl, but with 4 per cent. citric acid solution. After six weeks growth at 37°C., the reactions, estimated quantitatively, were as follows:—

<i>B. typhosus</i> : acid	(= 0.5	per cent. normal KOH).
<i>B. enteritidis</i> : alkaline	(= 4.25	„ „ HCl).
<i>Bacillus</i> 'L' : acid	(= 3.25	„ „ KOH).
<i>B. coli</i> : acid	(= 6.0	„ „ KOH).

Fermentation reactions in sugar media. This method gave very constant results. To avoid irregularity of constitution, simple peptone-water media were used in each case, the formulae being those recommended by MACCONKEY and HILL.²⁷ DURHAM'S⁷ fermentation tubes proved very satisfactory for preliminary determinations as to gas production; all the results were checked later by the use of THEOBALD SMITH'S³⁷ fermentation tubes, incubation lasting seven days. Determinations at the end of this time showed that *Bacillus* 'L' corresponded very closely with *B. enteritidis* in its fermentative powers. The nature of these fermentative changes in sugar media has been recently carefully studied by HARDEN.¹⁶

Formation of Indol. KRUSE²² pointed out in 1894 that the presence of 0.25 per cent. of glucose in bouillon inhibited indol-production. THEOBALD SMITH,³⁸ later on, showed that a medium entirely sugar-free gave the most reliable results. The tests were made, therefore, according to the method outlined by him. A series of tubes of

sugar-free bouillon (PECKHAM'S²⁹ formula*) was inoculated, one each day for ten days; these were all tested at the same time by the addition to each tube of ten drops of chemically pure H_2SO_4 and 1 ccm. of a 0.01 per cent. solution of NaNO_2 .

Formation of H_2S . The medium used was that suggested by COUTTS⁴ as very favourable (WITTE'S peptone 10 per cent., saturated solution iron tartrate 0.1 per cent., slightly alkaline to litmus); time of incubation, seven days.

Thermal death point: Heating to 65°C for five minutes, or to 60°C for twelve minutes completely killed the cultures. Heating to 60°C for five minutes, was not sufficient to destroy the growth, many colonies appearing on plate cultures.

PATHOGENICITY OF BACILLUS 'L'

Guinea-pigs, weighing 300 grms. or less, were killed within twelve hours by intraperitoneal inoculation of one small loop of a sixteen-hour agar culture. Larger guinea-pigs were made very ill by a similar dose and showed marked symptoms of toxæmia, but eventually recovered. Bacillus 'L' was recovered in large quantities from the blood and organs of the dead guinea-pigs.

Killed cultures: 5.0 ccm. of a killed bouillon culture, five days old, failed to kill a young guinea-pig.

FEEDING EXPERIMENTS

Two small rabbits, A and B, weighing respectively 284 and 386 grammes, were used for this purpose. They were fed daily with cabbage or bread, which had been soaked in bouillon cultures of Bacillus 'L.'

Rabbit A, first fed January 10, 1902.

January 13. Marked diarrhoea.

„ 14. Refuses food, very ill; temperature subnormal.

„ „ Died at 3 p.m.

„ „ Autopsy at once. Peritoneum normal; ileum shows swelling of PEYER'S patches and solitary follicles; the large gland at the ileo-caecal valve was much swollen, and showed haemorrhagic points on its surface; spleen not swollen.

Cultures: Peritoneum, heart blood, spleen, gall-bladder, negative; ileum, bacillus 'L' in abundance; mesenteric glands and bladder, bacillus 'L' in pure culture.

* Beef muscle, 225.0 grms. Trypsin, 4.0 grms. NaCl, 5.0 grms. Water, 1000.0 c.cm.

Rabbit B, first fed January 10.

- January 15. Ill ; quiet ; temperature not raised ; diarrhoea.
,, 16. Very quiet ; diarrhoea increasing.
,, 17. Violent diarrhoea ; temperature subnormal.
,, 18. Died at 9.30 a.m.
,, 18. Autopsy at once. Peritoneum normal ; swelling of lymphatic
follicles of ileum greater than in rabbit A ; spleen not
swollen.

Cultures : As in rabbit A.

Both rabbits evidently succumbed to a toxæmia. The diarrhoea was continuous and seemed to exhaust the strength of the rabbits, as it did that of the patient. *Bacillus* 'L' was recovered, as in the case of the patient, from urine and faeces.

SERUM REACTION OF IMMUNIZED ANIMALS

Two guinea-pigs were immunized by intraperitoneal inoculation with increasing doses of *Bacillus* 'L.' The effects of the serum of one of these upon a number of bacilli, as compared with the effects of the sera of guinea-pigs immunized against other organisms, are shown in the following table.

TABLE VII

SOURCE OF SERUM	DILUTION	B. TYPHOSUS (B.T.A.)				B. ENTERITIDIS				B. PARACOLON				BACILLUS 'L'			B. COLI (ESCHERICH)		
		1/2 hour	1 hour	2 hours	1/2 hour	1 hour	2 hours	1/2 hour	1 hour	2 hours	1/2 hour	1 hour	2 hours	1/2 hour	1 hour	2 hours	1/2 hour	1 hour	2 hours
SERUM A ... (From guinea- pig immunized against Bacillus 'L'.)	1: 10	*	*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1: 50	o	*	*	o	o	o	o	o	o	o	o	o	+	+	+	+	+	+
	1: 200	o	o	o	—	—	—	—	—	—	—	—	—	+	+	+	*	*	*
	1: 500	o	o	o	—	—	—	—	—	—	—	—	—	*	*	*	o	o	o
	1: 1000	—	—	—	—	—	—	—	—	—	—	—	—	*	*	*	o	o	o
SERUM B ... (From guinea- pig immunized against B.typho- sus.)	1: 50	+	+	+	+	+	+	+	+	+	+	+	+	o	o	—	+	+	+
	1: 200	+	+	+	+	+	+	+	+	+	+	+	+	o	o	—	+	+	+
	1: 500	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+
SERUM C ... (From guinea- pig immunized against B.enter- itidis.)	1: 50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1: 200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1: 500	tr	*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1: 1000	o	o	tr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1: 2000	o	o	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SERUM D ... (From guinea- pig immunized against B.coli.) (ESCHERICH)	1: 100	tr	tr	tr	+	+	+	+	+	+	+	+	+	tr	tr	tr	+	+	+
	1: 200	o	o	o	—	—	—	—	—	—	—	—	—	tr	tr	tr	+	+	+
	1: 500	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	tr	+	+	+

+, positive. *, partial. tr, trace. o, no clumping. —, not tried.

It is rather remarkable that serum A should have had so marked an effect on *B. coli*. The readiness with which the latter bacillus is agglutinated is, however, well recognized; unfortunately, the serum had not been tested with *B. coli* before the guinea-pig was inoculated with *Bacillus* 'L.' Serum A evidently had very little influence upon *B. typhosus* or the two members of the *B. enteritidis* group.

IDENTIFICATION OF *BACILLUS* 'L'

From the cultural relationships described above, it seems justifiable to put *Bacillus* 'L' into the *B. enteritidis* group, in spite of the divergence of its reaction in milk. This seems the more reasonable when it is remembered that milk cultures of the *B. typhosus* group are not uniform, some becoming alkaline after a week or more of incubation, whereas the typical group reaction in milk is acid.

Of the bacilli described by KRUSE in FLÜGGE's *Micro-organismen*, there are four with which *Bacillus* 'L' is comparable: *B. icterogenes*, *B. paradoxus*, *B. monadiformis*, and *B. chologenes*. The statement 'does not coagulate milk,' made with regard to the first three of these, does not give information as to whether or not these organisms render milk alkaline. However, *Bacillus* 'L' is differentiable from the first, third, and fourth, in that the latter ferment lactose; from the second, because of the latter's close resemblance to *B. typhosus* on potato.

Bacillus No. 32 in GERMANO and MAUREA's¹² elaborate table is the only one found recorded with which *Bacillus* 'L' may be said to agree culturally; even in this case it is not stated by the writers whether or not milk was rendered alkaline. In view of the aetiological relationship of *Bacillus* 'L' described in the present paper, it is not unlikely that GERMANO and MAUREA's No. 32 was the infective agent in the supposed case of typhoid from which it was isolated; but that was before the days of the serum-reaction.

This study has fully confirmed the opinion expressed by CUSHING⁵ that whether the cultural characteristics of the *B. enteritidis* group are but temporarily acquired and their intermediate existence merely transient, may be a question of dispute; but that nevertheless the definiteness of their serum-reactions, their pathogenicity, and the constancy of their cultural features justify placing them in a separate group.

It may not be out of place to draw special attention to the papers by CUSHING⁵ and DURHAM⁹ which have been freely consulted during the present study; the former illustrating the possibilities of a complete study of a group of organisms; the latter combining theoretical suggestiveness with a useful working classification of the groups under discussion.

SUMMARY

1. Intermediate between *B. typhosus* and *B. coli* are a number of organisms whose cultural characteristics are so constant (chief among them being their power of fermenting glucose but not lactose, combined with an inability to clot milk), that they may well be classified together as forming the *B. enteritidis* group.
2. The fourteen cases hitherto reported, of fever clinically like typhoid, whose bacteriology and serum-reactions show that they were not caused by *B. typhosus*, have been practically all caused by members of the *B. enteritidis* group.
3. The present report adds another case to this series ; the aetiological organism, *Bacillus* 'L,' being very closely related to the *B. enteritidis* group.
4. A study of the cultural features of this group shows the importance of (*a*) recording the action of a given organism in lactose as well as in glucose media ; (*b*) observing milk cultures over an extended period of time, not less than two weeks.
5. The Neutral-red reaction in glucose media cannot be regarded as characteristic of *B. coli*, but is readily given by the members of the *B. enteritidis* group, organisms capable of producing an infection like typhoid fever.
6. A negative serum-reaction in cases clinically diagnosed as typhoid, rather than lowering the value of serum diagnosis, may have an important bearing on our knowledge of the aetiology of typhoid fever.
7. In cases, apparently typhoid, which give a negative serum-reaction with *B. typhosus*, the agglutination-test should also be made with available organisms of the *B. enteritidis* group.

In conclusion, the writer desires to express his thanks to Dr. A. S. Grünbaum for constant guidance during the study, and for many suggestions which have been followed in the work ; also to Dr. Caton for his kind permission to report the case ; and to Dr. Frederick Griffith, House Physician in the Royal Infirmary, for much assistance in procuring specimens for bacteriological study.

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NOTE UPON FUNGUS DEPOSITS IN UNFILTERED
WATER MAINS

NOTE UPON FUNGUS DEPOSITS IN UNFILTERED WATER MAINS

By RUBERT BOYCE

In 1899 I was requested by the Water Engineer of the City of Liverpool, Mr. JOSEPH PARRY, to examine and report upon certain deposits which had gradually formed in the aqueduct conveying the unfiltered water from Lake Vyrnwy to the filter beds at Oswestry, twenty-five miles distant. In consequence of this deposit, there had been a gradual falling-off of the quantity of water capable of passing through the pipe line in the twenty-four hours. It was therefore considered of primary importance to seek the cause of the retardation, and to suggest a remedy. There have been many well-recorded cases where deposits in unfiltered water have led to a very serious falling-off in the daily water supply, notable cases have occurred at Lille and Berlin. In these instances the water contained much iron, and the deposits were shown to consist largely of *Crenothrix polyspora* (well-pest), a fungus in whose gelatinous sheath iron becomes deposited as ferric oxide.

Not only do these deposits lead to a diminution in the capacity of the water mains, wells, and reservoirs, but by forming a sticky layer on the surface of the filter beds, they hinder the normal rate of filtration, and further, the particles of fungus may impart to the unfiltered water an appearance, which has been aptly compared to thin coffee, and which, without treatment, may render it useless for domestic purposes.

The question of the association of large masses of some special organism with fluid containing special constituents has of late attracted considerable attention, and in a previous number of the *Thompson Yates Laboratories Reports** I described the two species of 'Sewage Fungus,' *Sphaerotilus* and *Leptomitus*, which are to be found in dilute sewage waters, and which, by their bulk, may lead to the blocking of drains and to offensive secondary decomposition. Like the water fungus the sewage fungus has a gelatinous sheath in which oxide of iron is formed when iron is present in the dilute sewage, and therefore, like the *Crenothrix*, the colour of the *Leptomitus* or *Sphaerotilus* is often brown; but whereas when the sewage fungus dies the oxide is converted into a black sulphide with the production of the odours of decomposition. I have never seen in the fungus deposits in pure water the production of any bad-smelling compound, nor is there any black sulphide formed, no matter how long the deposit is kept; on the contrary, the odour is rather pleasant, and the number of

* *Sewage Fungi*, Rubert Boyce, vol. III, part I.

bacteria present are comparatively few. More recently attention has been drawn to the nuisance which the green algae may create in certain waters, an example being recently described in the case of Belfast Lough by Professor LETTS. Whether the blocking, which occurs in the coke or contact beds used in the various forms of bacterial treatment of sewage, may in part be due to the gelatinous material, which is associated with the very great production of zoogloea masses which occurs on and near the surface, I have not yet satisfied myself, but I have observed considerable blocking associated with the deposition of ferric oxide upon the coke, and it is probable that this ferric oxide is to a large extent dependent upon the action of the zoogloea masses.

It will be gathered from the above remarks that the study of fungus deposits, whether occurring in sewage or water, is of considerable scientific interest, because in both cases a very active and special metabolism is taking place, which, in the one case, leads to the purification of sewage by the destruction of albuminoid matter and probably also by the assimilation of sulphur, and in the other to the removal of dissolved iron, but in both cases the disadvantages outweigh the advantages, and the study becomes one of very practical importance. Before describing the nature of the deposit in the Liverpool unfiltered water, I will briefly indicate the main features of the water supply.

DESCRIPTION OF THE LAKE VYRNWY WATER SUPPLY

The watershed is on the east side of the Berwyn range of hills in North Wales, the geological formation silurian (slate rock and Bala ash), the soil consists, to a large extent, of peaty moorland, and the water drains by innumerable streams into the artificial lake known as Lake Vyrnwy. The lake holds an immense volume of water, it is five miles long and three-quarters of a mile wide at its greatest width, and its maximum depth is eighty-four feet.

The water passes from the lake through a fine straining copper gauge into the aqueduct, which consists, for the first two miles as well as for the final two miles, of tunnels driven through the rock and partly lined with brickwork, for the remainder of the twenty-five miles the water is conveyed through large iron pipes (forty-two inches internal diameter).

Arrived at Oswestry the water collects in a storage reservoir, and from thence passes direct to the filter beds.

From the filter beds the water is conveyed thirty miles by iron piping to Liverpool.

COLOUR AND NATURE OF THE WATER

The striking feature of the unfiltered water is its yellow tint, which varies at different seasons; the colour is removed to a considerable extent by the filtration. The water is very soft, it contains iron and manganese.

PLACES WHERE EXAMINATIONS WERE MADE

1. *Lake Vyrnwy*—
 - (a) Water of lake.
 - (b) Bottom of lake.
2. *Surface of straining gauge.*
3. *Water at intake and exit of aqueduct.*
4. *Deposit along sides of aqueduct at numerous points both in the brick culverts and in iron pipes.*
5. *Reservoir at Oswestry*—
 - (a) Water of reservoir.
 - (b) Bottom of reservoir.
6. *Surface of filter beds.*
7. *The filtered water.*
8. *The sides of the filtered water pipes.*

METHODS

In the case of water, I have found by far the best method of examining solids in suspension to be to centrifugalize definite quantities and to read off the amount deposited in special centrifuge tubes. In this way it can be determined that the solids in suspension are more abundant in the lake at the origin of the aqueduct than at the Oswestry end, and that filtration removes the greater part.

In the case of the bottom of the lake at Vyrnwy and of the settling reservoir at Oswestry, I use a special suction apparatus, operated from the boat on the surface of the water. By this apparatus I have been enabled to thoroughly examine the bottom of deposits at very numerous points, and at depths varying from eighty feet to a few feet.

In the case of the aqueducts, I have scraped off the deposit from the interior of the iron pipes and brickwork when the water has been turned off.

CHARACTER OF THE DEPOSIT

Wherever it occurs the appearance of the material is very characteristic, it very closely resembles a fine coffee ground deposit, it has a golden brown colour, but in any quantity it is a very dark brown. It is heavy, and falls to the bottom. I have found it distributed over considerable areas of the bottom of the lake, in some places more abundant than at others. A very considerable deposit of the coffee ground material has formed where the compensation water passes from the lake to form the River Vyrnwy. On the bottom of the settling reservoir at Oswestry, especially near the mouth of the Vyrnwy Aqueduct, there is also a large deposit.

The appearance of the deposit coating the Vyrnwy Oswestry Aqueduct is more characteristic, a uniform fine coffee ground material, almost black in colour, coats the iron pipes uniformly all round, varying from $\frac{1}{8}$ -inch to $\frac{1}{2}$ -inch. In the case of the brick culverts only the portion of the brickwork covered by the water is coated. In the iron pipes there are innumerable limpet shaped incrustations of various sizes from a split pea to two or more inches in diameter, they are $\frac{1}{4}$ -inch to one inch in height. They are covered by the deposit, when cut into they are composed of alternate layers of red and black rust-like material. The odour of the deposit is characteristic, it is more metallic and aromatic than that of freshly turned earth.

After drying and incinerating a very abundant ash is left. Iron is present in large quantities, and the yellow brown colour is, no doubt, due, in this instance as in other cases, to the presence of this metal. Manganese is also present, and the dark black colour is probably due to it.

MICROSCOPIC APPEARANCES

Wherever the deposit occurs in the unfiltered water, whether in Lake Vyrnwy, or along the aqueduct, or at Oswestry, the appearances under the microscope are essentially the same. The bulk of the deposit consists of *irregular flakes of a soft material of a characteristic golden brown colour*, numerous silicious particles, a considerable amount of vegetable debris, but remarkably few green algae of any kind. The absence of green algae is very noteworthy, it is quite unlike what occurs in river water, and is no doubt related to the composition of the water. The higher green forms of aquatic plants are also conspicuous by their absence from the lake. The number of bacteria present are comparatively small, and this is corroborated by the culture experiments which have been made by me upon an extended scale. The golden brown irregular masses have imbedded in them or sticking to them all kinds of debris. They are soft, because they can be flattened out under the coverslip, and they appear to be composed of some gelatinous material impregnated with the yellow oxide of iron. They often show no trace of structure, but flakes will be met with, which, on very careful examination with the high power, show passing through them one or more distinct hyphae. I have been able to observe these hyphae in the otherwise structureless superficial layers of the limpet-like incrustations of the iron pipe previously alluded to.

Fig. 1 (Plate II) shows very well, magnified one thousand diameters, an irregular golden brown mass. In this photograph it appears very difficult to relate the gelatinous mass to the hypha shown running through it, but in Photographs 2 and 3 of younger fragments equally magnified one thousand diameters, the gelatinous material assumes much more the character of a sheath to the hypha. When the material which clings to the copper gauze screen, previously referred to, is examined, or when the deposit obtained by centrifugalizing the water of the lake is examined,

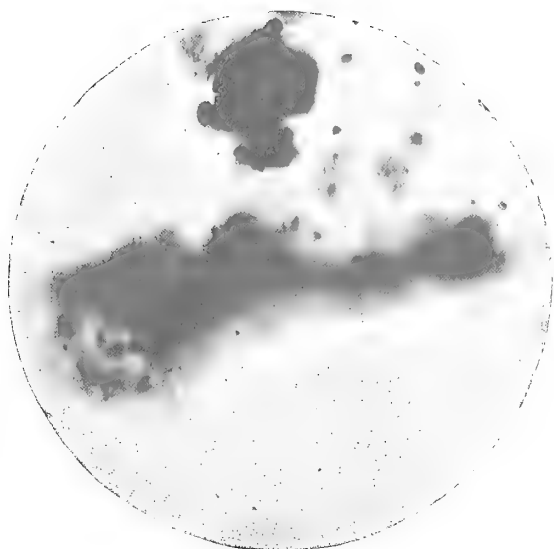


FIG. 1



FIG. 2

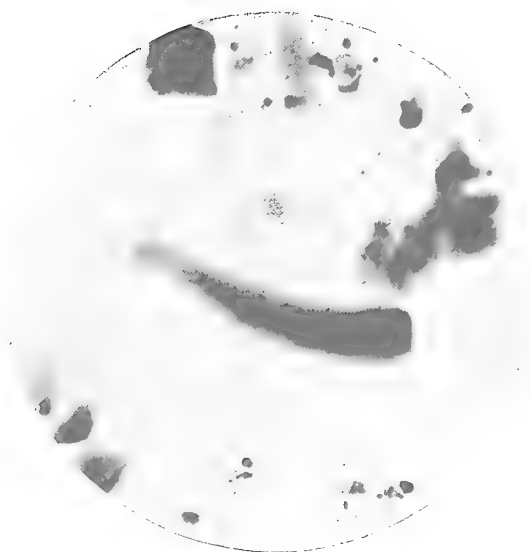


FIG. 3

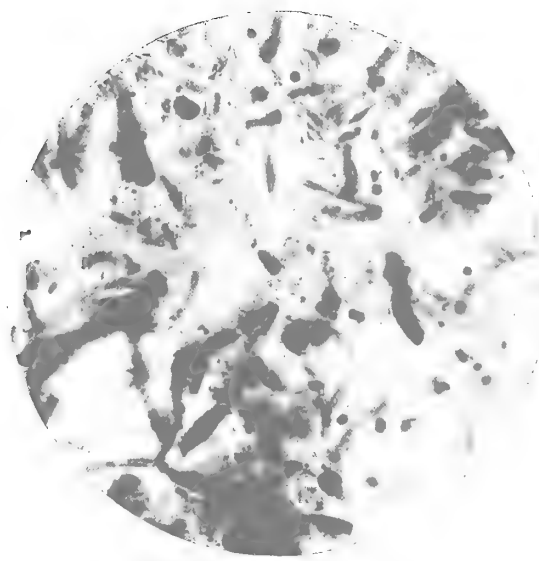


FIG. 4

the hyphal character of the flakes is much more evident, and long unbranched threads can be seen possessing a greater or lesser amount of sheath. Professor CAMPBELL BROWN has drawn attention to this organism, and there is every probability that it is a gelatinous iron forming thread fungus of the *Cladothrix*, *Leptothrix*, *Crenothrix* group. In none of the deposits which I have examined, have I been able to detect active growths of the organism, it almost invariably occurs in the short, irregular, broken fragments or flakes described above, a group of such fragments is well seen in Fig. 4 embedded in a gelatinous matrix. A most remarkable feature is that the gelatinous material appears to increase with age; it is not on the actively growing filaments that it is most abundant, it is thickest on the broken and apparent dead fragments.

The history of the deposit would appear to be as follows:—

The iron fungus grows in some parts of the watershed, fragments are carried with other debris into the lake, and they fall to the bottom, other larger and younger threads float in the water of the lake and are caught on the copper gauze strainer, innumerable small fragments however pass through the strainer into the aqueduct and gradually collect at the sides and form a coat on brickwork, wood, or iron of the pipe. Their gelatinous nature facilitates their adhesion, and they entangle with them all kinds of other debris as sand, diatoms, etc. With age the lower layers of the deposit appear to become more and more compressed and to lose all resemblance of fungus origin.

Not all the particles are deposited on the sides of the pipe line, some pass on with the water into the Reservoir at Oswestry, and no doubt also particles are continually being detached from the surface of the aqueduct. *But the filters remove them.* There is no doubt that the gelatinous sheath makes it exceedingly difficult for them to be carried through the sand of the filters. The result is that there is no deposit in the filtered water pipes.

As mentioned at the outset, Lille and Berlin were greatly troubled by deposits produced by *Crenothrix*, in the case of Berlin it is stated that they found a sediment many inches deep in the reservoirs.

RELATION OF THE ORGANISM TO THE IRON

There are numerous examples of bacterial activity leading to the oxidation of dissolved iron, and a group of organisms have been called 'Iron' and 'Manganese' bacteria from their power of producing the oxide of these metals from the soluble forms dissolved in the water. To this group *Crenothrix* and allied thread forms, including the present one, belong, and if WINOGRADSKY is correct, the filament absorbs the soluble iron salt and deposits the oxide in its sheath.

ZOPF regarded the process as mechanical. It appears to me to be exceedingly difficult to interpret the abundant formation of gelatinous material, and the abundant oxidation in the oldest filaments. The presence of the fungus is not peculiar to the

iron pipes. The same deposits occur in the reservoirs, in the brick culverts, and occurred at Berlin in the reservoirs, it is associated with the iron present in the source of supply.

Its presence in the water is beneficial in so far as it helps to remove by oxidation the dissolved iron, which assists to impart the yellow colour to certain waters ; and the black coffee ground deposit itself, containing as it does, abundance of iron and manganese oxides will probably act in the same way.

The disadvantages, however, of any substance which tends to accumulate on the sides of the pipes, and, therefore, to diminish the flow through them, are greater than the advantages, and a remedy has to be sought.

MEANS OF PREVENTION

Filtration. Long experience has proved this to be one of the most effective means of removing particles in suspension.

Screening. Where filtration is not available a great deal may be accomplished, as has been shown by the Liverpool Water Engineer, by the use of fine copper gauze screens which keep back the larger threads and other debris.

In so far as the dark colour of the unfiltered water is due to the presence of particles of the dark red fungus held in suspension, it is clear that the mechanical filtration will help to remove the colour. But the dark tint remains after the particles have been removed, this colour being due, partly to the presence of some organic colouring matter, and partly to the presence of some iron compound in solution. The removal of this colour is a very difficult matter, and the following processes have been recommended, all of which tend to bring about the oxidation and deposition of any iron compound, as well as the oxidation of any organic colouring matter :—

- (a) Passing the water over coke towers in order to cause oxidation of the iron. This process has been used with great advantage on a small scale, and I have myself demonstrated its power of removing the yellow colour from water.
- (b) Allowing the water to fall in a rain for three or four yards in order also to bring about oxidation.
- (c) STECKEL's method by the addition of lime in order to decompose the soluble carbonate of iron and form the oxide.
- (a) Addition of powerful oxidizers such as ozone, hydrogen peroxide, or filtration through filter beds constructed especially of oxidizing materials.

The disadvantage of any precipitation method is that a precipitate is formed which must be removed by immediate filtration or settling. The combination of lime and filtration (STECKEL's patent) has proved efficacious for small supplies.

SULPHIDE PRODUCING ORGANISMS

SULPHIDE PRODUCING ORGANISMS

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The bacterial method of sewage treatment having brought into prominence the action of the septic tank, it was thought that a contribution to the study of the bacteria which give rise to the black colour of the sludge might not be without interest.

Substances examined. Sludge of septic tanks, faeces, black mud and water, and decaying vegetable matter were examined for the presence of these bacteria.

Methods. For the isolation of the organisms, some of the material was added to a flask containing 100 c.cm. sterile water; 1 c.cm. of this dilution was added to a second flask, etc. Plates were made of nutrient agar with 1 c.cm. of each dilution, and incubated at 37° C. Further, tubes of sterile ferro-peptone medium (peptone water containing $\frac{1}{2}$ -1 c.cm. saturated solution tartrate of iron per litre) were at once inoculated with original material, and incubated at 37° C for twenty-four hours. Dilutions of these cultures were then made, plated, and incubated as before.

By the latter method the stronger sulphide producers were more readily obtained, as they multiply very rapidly, apparently suppressing other bacteria.

The colonies thus obtained, differing in so far as macroscopic and microscopic appearances indicated, were tested for sulphide producing properties. And one might mention here that while differences in appearances of colonies are certainly of service in separating different bacteria, yet the same organism may present several strikingly different appearances in its colonies according to age, situation, or other unknown conditions.

The following method of testing for sulphide formation was ascertained by experiment to be the most valuable. A solution of ten per cent. peptone in tap-water is made alkaline by the addition of 20 c.cm. normal potassium hydrate per litre. A minute quantity of flowers of sulphur is added, and 1 c.cm. saturated solution of tartrate of iron per litre. It is then at once poured into tubes before precipitation of the iron can occur. In the neck of each tube is suspended between the plug and the glass a strip of filter paper. Sterilization is done for twenty minutes on each of three successive days in a steam sterilizer at 100° C. On inoculation with the various colonies the tips of the filter paper are moistened with sterile solution of lead acetate, and so hung as to be secure from contact with the medium. Incubation is

done at 37° C. In this way the formation of any sulphide is indicated by the appearance of the black sulphide of iron in the solution ; while sulphide of hydrogen or volatile sulphides cause also blackening of the lead paper.

Most of the bacteria isolated were identified as sulphide formers before their richer production in ten per cent. peptone was known. Two per cent. peptone had previously been used, standardized at + 10 and + 14 to phenolphthalein (+ 10 indicates acidity to the extent of 10 c.cm. normal acid per litre of medium, with phenolphthalein as indicator ; - 10 indicates alkalinity to the same degree). Hydrochloric acid and potassium hydrate were used in standardizing. In titration 10 c.cm. medium was added to 40 c.cm. distilled water, boiling hot, for each test ; $\frac{n}{20}$ potassium hydrate being used for neutralizing. A faint rose pink colour was accepted for the neutral point.

For purposes of identification two per cent. peptone is probably almost as reliable as ten per cent., for even those organisms, which form very little sulphide in ten per cent. peptone, yield it also in two per cent. On the other hand, several which form very little in two per cent. peptone yield it richly in ten per cent. peptone.

Various methods have been followed by investigators in the isolation of hydrogen sulphide producing bacteria. Lead carbonate is recommended by BEIJERINCK,¹ added to slightly alkaline ordinary 'fleisch' gelatine or agar in sufficient quantity to whiten the media. He spreads the material for examination on the surface of the poured plates. The hydrogen sulphide producing colonies manifest themselves by their dark appearance against the white surface, the darkening being due to the deposit of lead sulphide.

A salt of iron instead of lead has been used and recommended by STAGNITTA-BALISTRERI,² FROMME,² and others.

Both of these methods have been tried in this work, and have not been found reliable. Sulphide forming colonies grow excellently on the ferro-agar plates, but the blackness generally appears slowly, sometimes not at all. The white lead agar plates are still less favourable for the formation of sulphide, and unfavourable even for the growth of some strong hydrogen sulphide producing bacteria.

PAKES,³ in discussing methods of demonstrating the formation of hydrogen sulphide, states that he has found satisfactory a salt of iron or neutral lead acetate in three per cent. peptone water. This appears remarkable ; for in experimenting with lead acetate it has been found that, with the addition of $\frac{1}{2}$ c.cm. saturated solution of lead acetate per litre to two per cent. peptone, there is absolutely no blackening of the medium after prolonged incubation. This salt, indeed, markedly inhibits the growth of the organisms.

Sodium nitroprusside has been used by STAGNITTA-BALISTRERI, but with unfavourable results.

Lead paper suspended in the necks of the tubes, and preferably kept moist, has found greatest favour as an indicator. RUBNER⁴ mentions, as an objection to it, the fact that mercaptans form with lead acetate a yellow colour, gradually becoming dark, which might lead to mistaking this evidence for that of weak and slow hydrogen sulphide production. He prefers an organic salt of iron in fluid medium.

Factors influencing sulphide formation. These may be considered as follows:—

- (a) Composition of media.
- (b) Reaction.
- (c) Temperature.
- (d) Oxygen.

A. COMPOSITION OF MEDIA

This is the most important consideration for the successful demonstration of sulphide formation. Peptone was observed by PETRI and MAASSEN⁶ to be a very favourable ingredient, and to be more efficient in more concentrated solutions. APPEL⁵ and PAKES³ speak of it favourably. BEIJERINCK and some others do not mention it. STAGNITTA-BALISTRERI² claim equally good results in peptone bouillon and peptone-free bouillon. They were unable, however, to obtain positive results with a number of organisms which PETRI and MAASSEN found to yield hydrogen sulphide freely in peptone solution.

Experiments performed with the organisms presented in this article confirm the observations of PETRI and MAASSEN. Bacteria, which in peptone-free bouillon yield sulphide slowly and in small amount, may produce it freely in two per cent. peptone, more richly in five per cent., and yet much more in ten per cent. peptone.

PETRI and MAASSEN suggest that the efficiency of the peptone depends upon the loose combination of at least a part of its sulphur.

This unstable combination is evidenced by the fact that the ferro-peptone medium, heated in the autoclave at 120° to 130° for twenty minutes, yields some of its sulphur as sulphide of iron. With longer heating more sulphide is formed. Sulphide is formed likewise from peptone solution not containing iron, by similar heating, as is indicated by the giving-off of hydrogen sulphide after the addition of dilute acid. Further, treatment of peptone with dilute solution of potash ($\frac{n}{3}$), in the cold, liberates sulphur as sulphide.

Beef extract, on the other hand, does not give up its sulphur nearly so readily through such agencies.

The excessive formation of sulphide in ten per cent. peptone is probably not accounted for merely by the increase of the sulphur supply; for the increase, in some instances, of hydrogen sulphide and many other noxious compounds formed is too

great to be accounted for in this way. Concentration of meat extract does not cause increase of sulphide formation; two per cent. and ten per cent. Liebig's extract are alike unfavourable media. Perhaps this may be due to an extent to a deficiency of sulphur in the extract.

Flowers of sulphur in minute quantity, added to two per cent. peptone, clearly contributes to the amount of sulphide formed. This is quite equal to the amount formed in five per cent. peptone, although very much less than in ten per cent.

The addition of sodium thiosulphate makes little difference. Sodium sulphite, except in very minute quantity, inhibits sulphide formation.

Glucose added to the medium renders it more favourable for production of hydrogen sulphide by most of the tabulated organisms. This was determined as follows:— To a part of a solution of two per cent. peptone, containing 1 c.cm. tartrate of iron per litre, was added a minute quantity of flowers of sulphur; to a second part, the same amount of sulphur and one-half per cent. glucose, with a quantity of calcium carbonate to neutralize acid formed; the remainder was left unaltered. Each of these three media was inoculated as usual in tubes with the various organisms, lead paper being introduced, and incubated at 37° C. New sterile lead papers were introduced from day to day.

It was observed that the medium containing the glucose was generally less darkened by the sulphide formed, at least by the more active organisms, than the non-glucose containing media. But on the other hand the amount of hydrogen sulphide escaping from the tubes was with some organisms much greater with the glucose containing medium; for the lead papers introduced from day to day immediately became black in these instances, whereas this did not occur with the non-glucose containing media.

The less degree of blackness in the glucose media was probably due to the fact that the insoluble calcium carbonate in the bottom of the tubes did not sufficiently neutralize the medium to permit of the ready deposit of the sulphide of iron.

Organism No. 12 (see table appended) affords one of the best illustrations of the favouring action of glucose. In two per cent. peptone with or without free sulphur its powers of hydrogen sulphide formation are very slight. After seven days incubation the liquid of these media was scarcely at all darkened, the main evidence of sulphide formation being a slight black deposit in the bottom of the tubes, and a very moderate blackening of the lead papers. But in the glucose medium all of the fluid soon darkened, and each lead paper introduced from day to day blackened immediately. That introduced on the seventh day became blacker in a few minutes than any of those which had been suspended for days over the non-glucose containing media. Nos. 8, 10, 11, 13, also show very marked increase in glucose medium.

Such increase continues only so long as calcium carbonate remains in the tube. With its exhaustion the fluid loses what blackness it possessed, and new lead papers

no longer become blackened. In such cases the medium has become markedly acid to litmus.

Egg albumen and blood serum were proved by ZÖRKENDÖRFER and PETRI and MAASSEN, to be favourable for the production of hydrogen sulphide by bacteria. APPEL has obtained the formation in sterile urine, after the addition of flowers of sulphur.

B. REACTION

The reaction of the media was discovered to be a matter of considerable importance in the interest of sulphide formation. In order to ascertain the most favourable point of reaction, different degrees of alkalinity and acidity were selected in two per cent. peptone water, with phenolphthalein as indicator, as follows: - 6, - 3, 0, + 3, + 6, + 8, + 10, + 12, + 14, + 16, + 18, + 20, + 24, + 27, + 30.

It was observed that the organisms selected certain points at which the blackening occurred most rapidly and deeply. These points ranged, as a rule, from + 10 to + 16, differing with different organisms and, indeed, varying slightly from time to time with the same organism.

The importance of reaction in the cultivation of bacteria is well known. But it is of yet greater importance for the formation of sulphide; for a peptone tube may be very cloudy with the growth of a strong sulphide builder, in a too acid or too alkaline medium, with little or no blackening of the fluid or lead paper. Not infrequently, however, with the lapse of days, sometimes weeks, the medium seems to be so altered that sulphide formation commences. One has to consider also the action of acidity in preventing the precipitation of the sulphide of iron; for while, say, + 12 shows marked blackness of paper and fluid, a considerably higher degree of acidity may present as much blackness on the paper, but the fluid be almost or entirely free.

In ten per cent. peptone water the reaction is of much less consequence for the strong sulphide formers. The additional energy imparted to their activity in this medium evidently enables them to overcome the retarding influence of unfavourable reaction. It must not, however, be neglected even in ten per cent. peptone, especially in the case of weaker sulphide builders.

A note on the indicators used may not be out of place here. The end reaction of phenolphthalein is very delicate in peptone solution. A two per cent. solution of peptone in tap water is acid to phenolphthalein to the extent of about 10 c.cm. normal acid per litre, and slightly alkaline to litmus; a ten per cent. solution is acid to the extent of about 45 c.cm. normal acid per litre to phenolphthalein, but of nearly the same alkalinity to litmus as the two per cent. solution. Some substances in peptone evidently respond very differently to the two indicators; and neither of them appears reliable for the standardizing of these two solutions at a common point

of reaction. Otherwise the reaction most favourable for the formation of sulphide in ten per cent. peptone is much more acid to phenolphthalein than the most favourable reaction in two per cent. peptone; being about + 25 in the former, and + 1 to + 16 in the latter. Now, if these two reactions be interchanged in the two media, *i.e.*, the ten per cent. be reduced to + 1 and the two per cent. be increased to + 25, the sulphide forming power is almost destroyed in the latter, and much reduced in former. On the other hand, with litmus, the solution of two per cent. peptone in water, which is very favourable for sulphide production, is slightly alkaline; whereas the ten per cent. solution, nearly equally alkaline to litmus, requires the addition of about 2 c.cm. normal potassium hydrate per litre to permit of the most rapid production of sulphide. That is, the point of best reaction is much more alkaline to litmus in ten per cent. peptone than in two per cent.

A similar difference between the two indicators is illustrated by the fact that some of the bacteria described in the appended table in two per cent. ferro-peptone medium, while forming sulphide freely, induce a marked increase of acidity to phenolphthalein, but not to litmus. Thus, organism No. 1, after three days incubation, had induced an acidity to phenolphthalein of + 35; No. 2 and No. 9 an acidity of + 30; and No. 7 of + 25. But in all cases the medium was still alkaline to litmus. The controls of ferro-peptone water showed acidity of a little over + 10 to phenolphthalein.

It may be that the acids of the peptone, and those formed by the bacteria, are more compatible with their activity than hydrochloric acid. The fact remains, however it may be explained, that if the same acidity to phenolphthalein be attained with hydrochloric acid before inoculation sulphide formation does not proceed.

C. TEMPERATURE

Cold exerts a retarding influence on the formation of sulphide. A temperature of 37° C. proves very favourable, 25° C. less so, and 12° C. very unfavourable.

D. OXYGEN

The strong sulphide formers act quite as well anaerobically as aerobically. Some of them seem to prefer a limited amount of oxygen as is evidenced by the formation of sulphide in the depths of stab cultures and plate colonies, rather than at the surface. Indeed, when surface colonies begin to darken they not infrequently do so first in their depth, the surface remaining of its original colour. It is noteworthy that a number of these very powerful hydrogen sulphide forming bacteria, while giving rise to a deep black throughout the ten per cent. gelatine and agar tubes, fail entirely to form black colonies in identical media, in poured plates, under aerobic conditions. Attempts made to induce black formation anaerobically, according to BUCHNER's method, failed also.

NATURE OF THE SULPHUR COMPOUNDS

It would be most difficult to determine what combinations of the sulphur are effected by the bacteria from the various sulphur-containing bodies. It is clear that the blackening of the media does not indicate necessarily the formation of sulphide of hydrogen. It means simply that the sulphur is liberated in the free state or in combination with some element or radicle, double decomposition occurring thereafter with the formation of the black sulphide. There is no doubt, however, that sulphide of hydrogen is the preponderating compound formed. This is evidenced by its powerful odour, and the rapid blackening of the lead paper. After one or two days it may be evolved in such quantity by several of the bacteria that the medium is densely black, and the gas passing off in such quantities as to be detected by its odour throughout a large room. Lead paper held over the openings of the tubes is instantly blackened. A single stab of No. 1 in ten per cent. ferro-peptone agar caused, in thirty-six hours, such excessive gas formation that the densely black medium was split apart and driven upwards in the tube.

The odour of sulphide of hydrogen is always mingled with a number of other very disagreeable odours. Among these may be distinctly detected the sharp penetrating odour of mustard oils.

Mercaptans were detected after several days' incubation by their reaction with isatine. A drop of one-quarter per cent. solution of isatine in concentrated sulphuric acid, deposited in the necks of tubes inoculated with organisms No. 1 and No. 5, was changed in several minutes, or even less, from a yellowish red to a deep clear green. With several of the other active forms a reaction was less definite; the yellowish red colour disappeared and was succeeded by a faint faded green.

One form (No. 15) caused some darkening of the iron-containing media, but never was observed to blacken the lead paper, whether iron were present or not. The sulphide here cannot be sulphide of hydrogen. It must be of non-volatile character.

The remaining bacteria caused blackening of the lead paper too quickly to be accounted for without hydrogen sulphide. In every instance there was also some black deposit in the bottom of the tubes of media.

METHOD OF FORMATION OF SULPHIDE OF HYDROGEN

It has been widely accepted,^{6, 4, 5, 8} that the formation of sulphide of hydrogen by bacteria is due to the action of nascent hydrogen, evolved through the activity of the bacteria, upon free sulphur or its compounds, organic or inorganic.

DEBRAYE and LEGRAIN⁸ found that a number of bacteria fermenting maltose, with the formation of hydrogen, were capable of forming hydrogen sulphide when

maltose and free sulphur were added to the medium, but not otherwise. Hence they looked upon the formation of sulphide as an accident of fermentation.

On the other hand, BEIJERINCK¹ found that members of his 'aerobacter' group, which liberated free hydrogen as a result of fermentation, did not form hydrogen sulphide thereby when flowers of sulphur was added.

It was ascertained that at least a number of the organisms fermenting glucose form hydrogen gas in this process. The test used was the simple qualitative one of exploding with oxygen. The gas was obtained in the following manner :—Flasks of 150 to 200 c.cm. capacity were provided with closely fitting rubber corks, perforated for the passage of the shorter limb of U shaped tubes. These were sterilized and filled with sterile two per cent. peptone, containing one-half per cent. glucose. After inoculation the flasks of medium were incubated in an inverted position, the long limb of the U tubes extending to the full height of the flasks, and communicating by rubber tubing with another vessel, which received the fluid displaced by the gas formed. In this way from 20 to 75 c.cm. of gas were obtained from the various flasks, according to the fermenting power of the respective organisms.

The gas thus formed was collected in test tubes under water, and thoroughly washed in lime water, to get rid of the carbon dioxide present, which was formed in greater or less amount in every instance. Air was then admitted to the extent of about two and a half volumes of the remaining gas, and a flame applied to the mouth of the tube. The sharp, high pitched report of hydrogen was obtained with an almost colourless flame in the instance of organisms Nos. 3, 5, 7, 12, 13, 14. In the other instances, a low pitched report or strong puff resulted; the original gas burning down the tube with a blue flame. Of course hydrogen may have been present in these cases also in small amount; so also may marsh gas.

It has been already stated that glucose favoured the formation of hydrogen sulphide with many of the organisms. In no instance did it appear retarding in its influence. Now if nascent hydrogen, acting on flowers of sulphur, forms hydrogen sulphide, it is most probable that at least a certain amount of the increase resulting from the addition of glucose is attributable to the hydrogen evolved.

Sodium amalgam in distilled water, in which sulphur is suspended, gives rise to an excessive amount of hydrogen sulphide in a few hours at 37° C. The strength of the alkali formed at the end of the reaction was slightly above that of normal solution. A potassium hydrate solution of this strength effects no change in the sulphur unless heated, when it readily dissolves it.

But glucose favoured the formation of hydrogen sulphide by organisms also, which form no gas whatever from this sugar, viz., Nos. 1, 6, and 9. It would seem then that the chemical action occurring as a result of the addition of glucose, is favourable to the formation of hydrogen sulphide, apart from the evolution of hydrogen.

PETRI and MAASSEN⁶ are the strongest advocates of the theory of the formation of the sulphide of hydrogen through the action of nascent hydrogen. In support of their view they mention the extensive reducing processes occurring through the agency of bacteria, *e.g.*, reduction of nitrates to nitrites, to ammonia; the decolourization of litmus and indigo; the reduction of ferric- to ferrous-salts, and invert sugar to mannite. They assert that they have been able to derive sulphide of hydrogen through the action of nascent hydrogen upon all sulphur compounds which yield it, through the activity of bacteria; and that sulphur compounds which have not yielded their sulphur to nascent hydrogen, in their experiments, have not yielded it either to the action of bacteria. Yet it seems scarcely justifiable to conclude that sulphide of hydrogen is therefore formed, in general, through the action of nascent hydrogen as such.

The reduction processes of bacteria are probably very complex, and can well be conceived of as proceeding quite independently of nascent hydrogen.

The fact that a number of sulphur compounds yield their sulphur neither to nascent hydrogen nor to bacterial action does not prove that, in some other sulphur compounds, from which bacteria are able to produce sulphide of hydrogen, they do so through the selective energy of nascent hydrogen as such, even though nascent hydrogen be capable of effecting the combination; for the union of sulphur and hydrogen can be regarded as occurring in other ways through energy imparted by the living organisms. It has been observed by WINOGRADSKY¹⁰ and others¹² that certain fungi are capable of taking up hydrogen sulphide in large quantity, separating the sulphur from the hydrogen and oxidizing it. Just how these organisms are capable of dissociating the hydrogen from the sulphur, and of adding oxygen to the sulphur, would be difficult to say. It would also be difficult to say just how an organism can effect the chemical union of hydrogen and sulphur.

BEIJERINCK⁹ has isolated an anaerobic organism, which operates, he believes, in the depths of polluted waters, and which is capable of reducing calcium sulphate directly; a process which nascent hydrogen is incapable of accomplishing.

It may be that nascent hydrogen plays a more or less important part in the production of sulphide of hydrogen. On the other hand, as already indicated, other factors whose nature we do not understand, may operate without its assistance.

In addition to the formation of sulphide of hydrogen from proteid, sulphur and lower sulphur salts, may be mentioned a process regarded as occurring in nature, which has been investigated by HOPPE-SEYLER¹² and others, *viz.*, the fermentation of cellulose with the formation of equal parts of hydrogen and methane. According to HOPPE-SEYLER, the marsh gas reacts at once with calcium sulphate, if present, according to the equation.



DISTRIBUTION

The faculty of producing sulphide in a greater or less degree appears to be very general among bacteria. One must be guarded in stating that any individual form does not produce sulphide, for it has been observed that this property may vary somewhat in any organism, even a very strong sulphide builder, from time to time in the same medium. And, as already mentioned, an organism which cannot form sulphide from one substance may be capable of doing so from another.

PETRI and MAASSEN examined thirty-seven pathogenic bacteria, all of which proved capable of forming sulphide in a greater or less degree.

They ascertained that animals inoculated with *Rotblauf* bacillus acquired symptoms of hydrogen sulphide poisoning. Sulphide of hydrogen was demonstrated in the fresh blood and organs of such animals. Similarly it was detected in the subcutaneous tissue of an animal inoculated with bacillus of malignant oedema. The anaerobic pathogenic bacteria were found by them to be the most active in the formation of this gas.

In nature BEIJERINCK regards the anaerobic reducer of sulphates as producing more hydrogen sulphide than any other form. He places also bacillus coli in the front rank of hydrogen sulphide builders, partly because of its individual power and partly because of its wide distribution. STAGNITTA-BALISTRERI and RUBNER found bacillus coli to produce less than the typhoid bacillus. The appended table, in which the organisms are arranged approximately in order of sulphide producing activity, indicates that seven of them produce more sulphide than the forms of coli included. The activity of these coli forms differs among themselves. No. 8 is decidedly a stronger producer than No. 11. Both of them are stronger than the typhoid strains examined. The laboratory culture of the ESCHERICH form, on the other hand, proved weaker in production than the typhoid forms. The question of the distribution of coli is yet a debated one.

It will be observed that the majority of the active sulphide builders have been obtained from sewage and faeces. Several, however, have been derived from black mud or water. These several sources presented a common feature, viz., an exceedingly black appearance with sometimes an emission of unpleasant odours. The black formation apparently is an incident in the putrefactive process.

In all places it is not improbable that the black putrefactive processes result from faecal contamination. This suggestion is rendered more probable by the fact that all the active sulphide builders isolated yield acid and gas or acid in MACCONKEY's taurocholate glucose broth¹³ at 42° C, which he considers as strongly indicative of intestinal origin. It will be observed in the table that those organisms obtained from areas of decaying vegetation are, with one or two exceptions, weak sulphide producers. This is what one might expect from the character of the food supplied them, and from the appearance of the disorganizing matter.

DIFFERENTIATION OF BACTERIA ISOLATED

Glucose, lactose, saccharose, litmus milk and gelatine have mainly served for the differentiation of the organisms isolated. Mannite is generally fermented by those organisms which ferment glucose; but in one or two instances it has been of some differential value. Glycerine has been of value in distinguishing coli from closely related forms.

The media were constituted as follows:—glucose, .5 per cent. in two per cent. peptone water; lactose, saccharose, mannite, and glycerine, each one per cent. in two per cent. peptone water. All these contained litmus as indicator of acid formation, and Durham tubes to aid in the detection of gas formation.

The bacteria are not arranged according to relationship, but as before mentioned, according to power of sulphide production, so far as could be indicated by the rapidity and intensity of the blackening of media and lead paper, and the strength of odour evolved. Nos. 1-9 are very strong, 10-12 are strong in ten per cent. peptone, 13-14 moderately so, and 15-21 are weak. The first nine are capable of blackening a favourable medium in twelve hours.

It will be observed that several of the organisms form very little gas in saccharose. It might be suggested that the smallness of the amount is due merely to a variation in the fermenting power, and not to be regarded as a distinguishing feature of an organism. It can be stated, however, that during the months that these bacteria have been under observation, the amount of gas formed has never been greater. The weakness of the fermentation is also suggested by the absence of acid formation. Another constant and peculiar feature appears in No. 5, viz., rapid fermentation of lactose sugar, with formation of acid and gas, side by side with the marked acidifying of milk, without the formation of clot—even after two months incubation. Nos. 15 and 21 are likewise to be distinguished by the more luxuriant growth, and the excessive bluish green fluorescence of the former on all forms of media. No. 21 shows a very little fluorescence on some of the sugar media, and agar. No. 10 and No. 13 differ from each other in their growth on plates of MACCONKEY'S taurocholate lactose agar;¹³ No. 13 presenting about its colonies the clear rings peculiar to PFEIFFER'S bacillus capsulatus. No. 10 on the other hand forms colonies like bacillus coli of ESCHERICH, and as it also ferments glycerine and saccharose, it is probably bacillus lactis aerogenes. No. 8 differs from the B. coli of ESCHERICH in its decolourization of saccharose and in its fermentation of glycerine.

A number of other strong sulphide formers will be observed to be related to the coli group in their fermentation of sugars, e.g., 2, 3, 4, 5.

No organism has been regarded as a form of coli that does not form acid and gas with lactose, and acidify and clot milk.

In conclusion, I have to express my obligation to Professor BOYCE, who proposed this work, and to whom I am indebted for many kind suggestions, also to my laboratory associates and Dr. TITHERLEY for help given.

Organisms	Morphology	Glucose	Lactose	Saccharose	Mannite	Glycerine	Litmus Milk	Gelatin	Sources
1	Motile bacillus	A	D	A	A + C + P	L	Black mud, country road; black slime, country pond; black mud, Liverpool street.
2	"	A + G	D	A + G	A + G	...	A + C	L	Sludge, septic tank, Manchester; sludge, chemical precipitation tank, Manchester.
3	"	A + G	D	D + G*	A + C + P	L	Faeces; sludge, septic tank, Manchester; sludge, chemical precipitation tank, Manchester.
4	"	A + G	o	A + G*	o	...	A + C	L	Lip of septic tank.
5	"	A + G	A + G	A + G	A	N L	Faeces; sludge, Cameron tank, Manchester.
6	"	A	A	A	A	L	Raw sewage, Manchester.
7	"	A + G	D + G* alk. later	D alk. later	o	...	o	N L	Faeces; sludge, septic tank, Manchester; Cameron tank, Manchester.
8	"	A + G	A + G	D	...	A + G	A + C	N L	Faeces; sludge, septic tank.
9	"	A	D	D	A + C + P	L	Black garden mould.
10	"	A + G	A + G	A + G	...	A + G	A + C	N L	Black mud, Liverpool street.
11	"	A + G	A + G	o	...	o	A + C	N L	" "
12	"	A + G	o	o	A	N L	Black slime, country pond.
13	"	A + G	A + G	A + G	...	A + G	A + C	N L	Sludge, Cameron tank, Manchester.
14	"	A + G	D	A + G	A + G	...	A + C	N L	Faeces; sludge, Cameron tank, Manchester; precipitation tank, Manchester.
15	"	A	o	o	A + C + P	L	Sludge, septic tank, Leeds.
16	"	A + G	D	A + G	A	N L	Sludge, Cameron tank.
17	"	o	o	o	A + C + P	L	Black slime, country pond; black garden mould.
18	"	o	o	o	Alk.	L	Raw sewage; manure heap, field; black decaying vegetable matter.
19	"	o	o	o	o	L	Sludge, septic tank; sludge, Cameron tank.
20	"	A	A	A	A + C	...	Black slime, country pond; black garden mould.
21	"	A	o	o	A + C + P	L	Milk.

A, acid. G, gas. D, decolorization. C, clot. P, peptonization. L, liquefaction. *A few bubbles only in Durham tubes.

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A NEW NITROMETER FOR THE CLINICAL ESTIMATION
OF UREA BY THE HYPOBROMITE PROCESS

A NEW NITROMETER FOR THE CLINICAL ESTIMATION OF UREA BY THE HYPOBROMITE PROCESS

By W. G. LITTLE, M.A., ABERD., M.B., C.M., EDIN.

The following instrument has been designed with a view to facilitate the estimation of urea for clinical purposes. Like all such instruments, it cannot claim to be absolutely accurate in the results given, but in clinical work, as carried on by practitioners in general practice, absolute accuracy is often of less importance than ready usefulness and economy of time and material. Perhaps it may be claimed that in both these latter respects this instrument is an improvement, always presupposing reasonable care on the part of the operator.

The ureometer hitherto in greatest vogue amongst the limited number of practitioners who consider it part of the day's work to trouble about urea estimation has been that of DOREMUS or, as it is termed in this country, SOUTHALL'S ureometer. The chief merit seems to be that it represents a combination of good qualities. It is small, portable, and handy, but when we come to try it for practical purposes these very characteristics will be found to constitute its most patent drawbacks. Being so small, it is apt to get lost or knocked about. It is really too easily handled. The temptation to use it without a stand, the necessity to manipulate it so freely in filling and cleansing are real disadvantages, as being apt to interfere with the accuracy of an experiment, and the accompanying glass pipette is not a reliable implement unless very carefully handled. Superadded to these dangers are the facts (by no means unimportant to the average modern practitioner) that half an ounce of hypobromite solution is required for each estimation, and, what is perhaps worse, much time is wasted after the successful experiment by having to set aside the whole apparatus until the froth clears and any reading becomes possible. And even then the same solution, having still a certain amount of nitrogen in suspension, becomes thereby unfitted for a second estimation should the first have failed through some slip of manipulation, which is by no means impossible or rather not improbable, as anyone can testify who has tried this instrument. In fact, as a matter of experience, one may say that special adroitness with the pipette is needed in order safely to insert the point and leave behind all the 1 c.c. of urine without allowing any air to follow, and at the same time to prevent the escape of any nitrogen bubbles into the open

bulb, if that be possible. There are also other disadvantages. The interior of the long tube, on which are the markings, is apt to become more or less opaque, and cleansing is not always easy, owing to the shape and the consequent difficulty of reaching and removing any deposits that may have been thrown down inside during or after the chemical reaction. The pipette with rubber appendage for projecting the 1 c.c. of urine is not very easily manipulated. The dangers have already been alluded to. One cannot be certain that some of the adjacent air has not at the same time been introduced which, of course, will falsify the reading at once. And no second or confirmatory experiment is possible with the same charge or instrument until after the lapse of a very considerable length of time, too considerable, in fact, for the patience of an average practitioner.

The initial cost of this instrument may be small, but having regard to the price of bromine (each capsule containing 2 c.c., or the equivalent needed for one charge of hypobromite, costs in the provinces sixpence or to a keen business man fivepence) one finds that this claim to superiority vanishes in the extra cost of raw material needed for any series of estimations.

In the case of other well-known instruments (*e.g.*, LUNGE's nitrometer), purporting to be scientifically accurate, the price bulk and rig-up may be said to preclude safe and ready use anywhere save in a Physiological or Chemical Laboratory, where time is no object, where there is plenty of table space or elbow room, and where the prolongation of a glass tube, one or more feet, into space is of little consequence, especially where no unhallowed domestics come periodically to disturb or dust, and where a little confusion is not derogatory to social propriety. For example, this nitrometer, as used in the Thompson Yates Laboratory, would, in a private consulting room, require a special table, corner, and cupboard, or, better say, a special room all to itself, so as to avoid the daily risk of being knocked to pieces, and surely it is no exaggeration to say that there are few places where it is oftentimes more necessary to estimate the nitrogenous output of a patient (or to do as much in that direction as possible) without delay or disturbance, than in a private consulting room. Besides, in the case of this nitrometer, the presence of so much elasticity in the form of india-rubber and of so many component parts, in a state of potential instability, and the fact of having to tilt and, perhaps, shake the double bottle in which the nitrogen is generated, all this is liable in the ordinary course of human imperfection to dislocate something, especially the rubber stopper, and not only spoil the experiment but do what is worse, namely, spill the caustic hypobromite solution on one's table, or garments, or about the room, a consummation too terrible to be contemplated as occurring in any well-regulated establishment, however scientifically inclined the head of the house might be. It is also, as in the other case, a very decided objection to have to touch the bottle with a warm hand at all after the introduction of the urine, and the slightest accidental pressure on the rubber stopper, in order to keep it firm

in its place, necessarily affects the reading on the burette, and is fatal to the success of any particular estimation.

And last, but by no means least, is the question of cost. What with so much rubber, three pieces of glass, and a special stand, the price must be considerable (in the lowest price list 12s. 10d. altogether), and even yet, despite other forms of prosperity, there is, medically speaking, none so rich as not to do him reverence who can help to reduce the price of modern scientific apparatus. One might in this instance have less reason to object if the graduated and stoppered burette included were available for titration, but, being constricted at *both* ends, it is not.

These two instruments may be taken as typical of many others which seem only more complicated, and therefore more liable to go wrong in one or other of the parts.

One is quite ready, of course, to admit that no instrument of the nitrometer or ureometer species which affects to give the volume of nitrogen per c.c. of urine, and admits of being adjusted to correct such distributing variants as barometric and thermometric conditions, can be simple or cheap. But the question may be asked, why take the trouble to estimate and record the volume of nitrogen on any given day or at any given place except in terms of a constant standard? No two places, no two days probably, agree. However refined the scientific appliances, the results given must always contain a certain error, unless corrected to a constant standard.

Therefore if we take the plea of scientific accuracy as an argument for the use of carefully regulated, but complicated and costly apparatus, and as against the use of the more amenable and thriftier process but less absolutely accurate readings of a simpler instrument, it will not hold good. With the most perfect instrument one ought to 'standardize' all the same, whilst with a simple and ready instrument, even if not absolutely accurate, one can record the reading, and, knowing the error of the instrument, make one correction suffice. This can be done at leisure, and then the result will be perfectly accurate.

All this could be done for readings from the Doremus, or SOUTHALL's instrument, did other considerations warrant its employment. It is also quite easy to do the same with this one, and to prove how very small the error of any particular reading is. The error is, in fact, so small that it may safely be neglected, for however important absolute accuracy at times may be in the clinical work of a general practitioner, time, patience, and material also must count for something, and it is in the hope of economizing all three, as well as of simplifying the application of this Hypobromite process (said to be the most accurate), that this instrument has been designed. (See plate III).

It consists of one piece of glass only, which is affixed by one or more rubber bands to an upright fluted stand of wood, which, being fixed into a small horizontal square also of wood, may be moved with the instrument in situ from place to place.

The shape and arrangement are best illustrated by the above diagram. It is necessary also to be provided with a rubber stopper valve of the 'Allenbury' feeding bottle (value 4d.), which fits into the opening in the upper zone of the pearshaped bulb as seen above. Also a pipette to hold at least 5 c.c., for introducing and withdrawing the hypobromite solution before and after an estimation and cleansing the bulb. Having charged with hypobromite solution, insert the stopper with the notch on the rim pointing downwards, for that corresponds with the position of the slit seen in the diagram, which admits the urine and acts as a valve to prevent escape of the nitrogen, and adjust so that the level of the coloured fluid rises to the first *mark* on the graduated tube (not the first number). This is most easily done by injecting air by means of the syringe. The urine is then projected on to the surface of the hypobromite solution through the slit seen in the diagram of the stopper by means of an ordinary vulcanite glass hyperdermic syringe. The nozzle of the syringe ought to fit with a fair amount of tightness into the opening of the stopper, which, being of india-rubber, is adaptable. Push the piston of the syringe slowly inwards, and the urine escapes quite easily on to the surface of the solution inside, and having withdrawn the syringe and the fingers from the bulb rotate the instrument gently from side to side, catching hold by the arch above. *It is thus not necessary to touch the bulb containing the mixture evolving the gas after the urine has been introduced.* The rotary movement gives the requisite amount of shaking to liberate all the nitrogen speedily. Then leave for a minute or more to cool, or if time be an object, rotate again to see that no more gas is given off, and the coloured fluid in the graduated tube no longer rises, and cool by means of a cold wet rag. Then read off.

This gives the volume of nitrogen in c.cs. for 1 c.c. of urine. From that it is easy to deduce the urea. There is, of course, a slight error in volume owing to the extra pressure of the column of fluid (distilled water with three drops of red ink) in the graduated tube. The extent of this error may be estimated thus and is so small that for ordinary purposes it may be neglected.

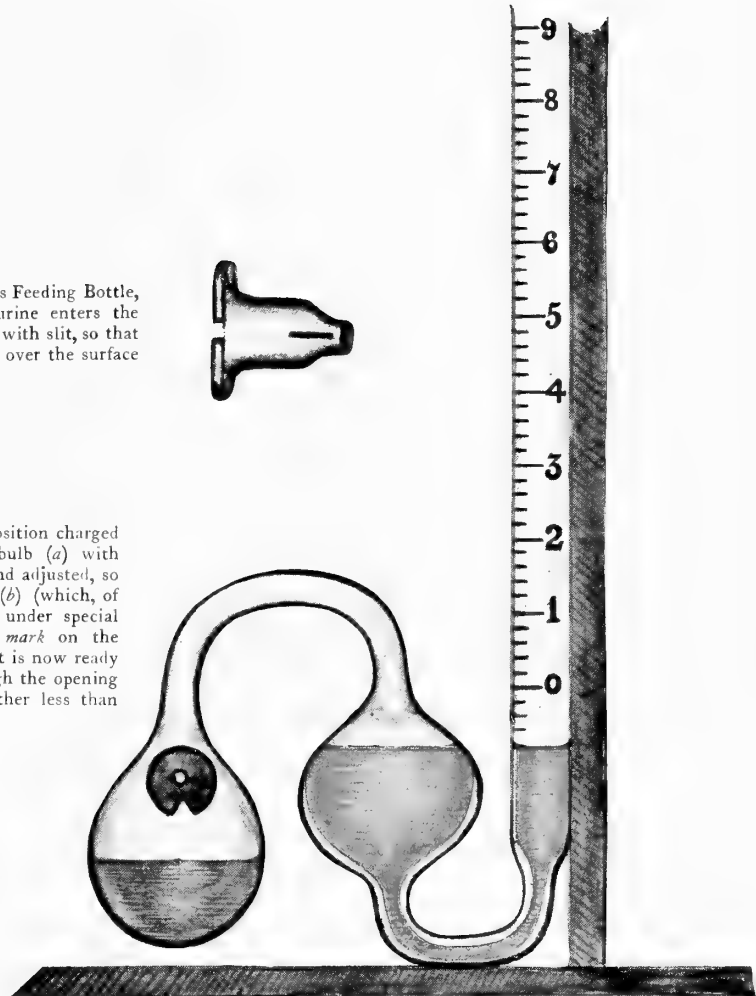
Suppose the volume of nitrogen read off for 1 c.c. of urine is 8 c.c. Measuring the difference between the level of the fluid in the tube and that in the bulb we find it say nine centimetres which, of course, means that the pressure on the gas in the bulbs exceeds the atmospheric pressure at the moment by nine centimetres of water. Then, according to BOYLE'S law, the volume of a gas varies inversely with the pressure. Air and nitrogen are almost equally compressible, so that the mixture may be calculated for as if it were only one gas. Thus, if V = correct reading and V_1 = reading as given by Nitrometer, and P = atmospheric pressure in c.c. of water. P_1 = pressure on mixture of gases = $(P + 9)$ in c.c. of water.

$$\begin{aligned}\therefore \frac{V}{P_1} &= \frac{V_1}{P} \text{ or } V = V_1 \left(\frac{P_1 + 9}{P} \right) = 8 \cdot \left(\frac{1033 + 9}{1033} \right) = \frac{8336}{1033} \\ &= 8.07 \text{ or an error of a fraction of a cubic millimetre.}\end{aligned}$$

Stopper valve of Allenbury's Feeding Bottle, shewing slit through which urine enters the bulb, also shewing notch in line with slit, so that it may be made to come directly over the surface of the Hypobromite solution.



Side view of instrument in position charged with Hypobromite solution in bulb (a) with stopper inserted in the opening and adjusted, so that coloured indicating fluid in (b) (which, of course, is never emptied except under special circumstances) is at the first mark on the graduated perpendicular tube. It is now ready to receive one c.c. of urine through the opening in the centre of the stopper. Rather less than half-size.



The error of volume arising from the superincumbent fluid is thus so small that it may safely be neglected for clinical work, and the reading given at once taken as the volume of nitrogen for 1 c.c. of urine, or for the quantity injected, whatever that may be.

Having read off, withdraw the stopper. The indicating fluid falls back to its original level. Remove the used-up solution with the pipette. Cleanse, recharge, adjust, and so on, any number of times, each time with fresh hypobromite solution.

It takes very little time to do any observation, and having used it once or twice it is quite easy with one tube of bromine or one ounce of fresh solution to do several urines (eight or nine), and if more than 2 c.c. be injected for a charge it is easy, without withdrawing the stopper, to inject a second c.c. of urine almost immediately with a view of corroborating the previous reading.

There is no trouble arising from the froth. It is possible to read off at once. Indeed, the character of the froth becomes a very delicate test for albumin in the urine. In dealing with albuminous urines SOUTHALL'S is *bors de combat*.

After an experience of several months one has had no breakages of glass. The glass may be made strong enough to withstand almost any reasonable strain, and as the instrument stands upright bound by one or more ordinary india-rubber bands to the column of the stand, it is never loose, and, therefore, the danger of being knocked about is reduced to a minimum. The floor of the wooden stand is about one foot square, and the height of the central fluted column is little over a foot high, so this gives a condition of very stable equilibrium and a small platform on which to rotate the tube and place instruments. It will protect an ordinary table, and may be moved about from one table to another. For a series of comparative estimations one has found this nitrometer especially useful.

The glass work has been very carefully and efficiently done by Mr. GRIGIONI, of Mersey Street, Liverpool.

EXTENSIVE FOCAL NECROSIS OF THE LIVER
IN EARLY TYPHOID FEVER

EXTENSIVE FOCAL NECROSIS OF THE LIVER IN EARLY TYPHOID FEVER

BY E. E. GLYNN, M.A., M.B. (CANTAB.)

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R. B., male, aged 28, a labourer, was laid up for four months in 1893, with rheumatic fever. After recovery, his health was good till the beginning of September, 1901, when he was attacked with 'shortness of breath and palpitation.' He ceased work on November 9, and was admitted two days later to the Liverpool Royal Infirmary under the care of Dr. BARR, with the physical signs of moderate mitral stenosis and regurgitation, and symptoms of failing compensation; pulse seventy-six and irregular. There was neither orthopnoea nor oedema of the feet. The tongue was slightly coated, bowels regular, there was no abdominal distension or tenderness; the liver and spleen were distinctly enlarged. There was no headache, no cutaneous eruption. The urine was normal, but diminished in quantity.

On the night of admission, November 11, the evening temperature was 100° F. During the next forty-eight hours, it varied between 99° and 97.2° . But on November 14, the morning temperature rose to 101° , the evening to 103.8° . During the next three days pyrexia continued with morning remissions (*vide* chart). The pulse averaged seventy-four, the respirations twenty-four; the bowels became confined. On November 18, he was delirious, the temperature rose to 104.8° . Next day it reached 105.9° , the pulse was one hundred and thirty-two; coma supervened, and he died on the ninth day after admission, six days after the onset of the pyrexia.

The main features of the autopsy were as follows:—

The heart was enlarged (weight 18 ounces), the cavities were dilated especially on the left side. There was moderate sclerosis of the mitral orifice.

The lungs were congested at the bases.

The liver (weight 52 ounces) was very pale, soft, and dotted irregularly with bright red spots varying in size from a pin head to a pin point.

The Peyer's patches and solitary follicles of the last three feet of ileum were greatly swollen; but without evidence of ulceration.

There was enlargement and congestion of the mesenteric glands.

The spleen was enlarged (weight 11 ounces) and rather tough.

The kidneys were pale and the brain normal.

lymph sinuses; certain of these were large and phagocytic, containing leucocytes in various stages of disintegration—a condition described by MALLORY. A few plasma cells were seen; multinuclear leucocytes and fibrin were practically absent.

No ulceration had occurred on the surface of the glands, but in the deeper parts there were a few patches of necrosis dotted with fragments of nuclei. The submucous coat contained several endothelial cells and lymphocytes, the latter mostly grouped around the engorged blood vessels. The muscular and peritoneal coats were normal.

The most striking feature in the mesenteric gland, apart from the general congestion, was extensive necrosis. The normal lymphatic nodules had almost completely disappeared, and were replaced by darkly staining granular masses filled with fragmented nuclei, in appearance not unlike early tubercular caseation. The lymph sinuses adjoining these masses were blocked with fibrin and endothelial cells in an early stage of necrosis.

The clinical history, the results of the autopsy, the microscopic appearances of the intestinal lesions, and the bacteriological examination, prove that the patient was suffering from chronic heart disease, and died from a virulent attack of typhoid fever. The duration of the attack was probably six days, beginning with the pyrexia on November 14; the slight temperature on November 11 is of no significance, for patients are often feverish during the first night in the hospital.

The absence of intestinal ulceration is additional evidence of the brevity of the attack, while the presence of some necrosis within the lymphoid tissue, a condition usually obtaining about the tenth day of the disease or later, does not prove that the disease was in the second or third week; but it must be regarded as a natural result of the virulent infection indicated by the temperature and cerebral symptoms.

The microscopical appearances of the liver were most interesting. Apart from early and uniform parenchymatous changes the organ was studded with areas of focal necrosis, which were so numerous that three or more often appeared in the field of a half-inch objective. The areas were usually slightly larger, sometimes much larger, than a human kidney glomerulus (Plate IV, Fig. 1). They were situated mainly at the periphery or in the centre of the liver lobule. Their shape was roughly round or oval, the margins being rather irregular. They consisted essentially of necrosing or necrosed cells—probably liver cells—detritus, and occasionally persisting strands of stroma mingled with numerous irregular nuclei, sometimes club shape, triangular, or fusiform; and often a few lymphocytes. These irregular nuclei were as a rule more conspicuous than the necrosing cells, giving the lesions a most characteristic appearance, and especially when a few lymphocytes were present, suggested the original misnomer 'lymphoid nodules.' This term is less incorrectly applied to certain areas of the periportal connective tissue densely infiltrated with lymphocytes, but in which there was neither necrosis nor irregular nuclei—a lesion met with in typhoid and other toxic diseases.

The red points noticed at the *post-mortem* were due to marked dilatation of the capillaries in certain lobules, combined with compression of the adjacent hepatic cells. These lesions being seldom associated with focal necrosis were perhaps the result of passive congestion, though neither their microscopic appearances nor distribution were typical; on the other hand, LONGRIDGE described marked localized dilatations of the hepatic capillaries in a case of focal necrosis, and FLEXNER produced dilatation and focal necrosis in the liver of rabbits by the inoculation of ricin in small quantities.

The hepatic capillaries were blocked at intervals with dense clusters of micro-organisms, which from their morphology and staining reactions, and on account of the fact that *B. coli* was not isolated from the spleen, must be regarded as typhoid bacilli. As there was no relation between the focal lesions and the groups of typhoid bacilli, their presence in such numbers was probably due to *post-mortem* invasion and multiplication.

Divergent views are held as to the composition of the necrotic areas, and the origin of the irregular nuclei. All writers agree that the hepatic cells necrose, but according to ORTH and others the nuclei arise from lymphoid cells. WAGNER holds that they are produced by the multiplication of hepatic cells. According to REED they 'owe their origin, in small part, to the disintegration of the nuclei of the liver cell involved, and in greater part to the presence of polynuclear leucocytes.' MALLORY maintains that invasion with polynuclear leucocytes is 'rare,' and also that the lesions are due to the 'obstruction of the liver capillaries by phagocytic cells derived in small part from the lining endothelium of the liver capillaries, but chiefly by embolism through the portal circulation of cells originating from the endothelium of the blood vessels of the intestines and spleen,' for the typhoid toxine produces a diffuse proliferation and desquamation of the endothelial cell which is, he believes, the 'essential lesion of typhoid fever.'

Polynuclear leucocytes were absent in my sections; there was no evidence that large endothelial cells were associated with the formation of focal necrosis, perhaps because the tissues had been fixed and hardened in spirit.

The exact causation of the lesion is also disputed. Observers agree that typhoid bacilli are not found in the necrotic area. Therefore, the lesions probably originate either by the action of the diffusible typho-toxin on groups of cells in which the resistance had been lowered for some reason or other, or in the manner which MALLORY suggests. But if the embolism of phagocytic endothelial cells be the main cause of the focal lesion, why are they not confined to the portal zone of the hepatic lobule, and why, as MALLORY himself asks, are phagocytic endothelial cells not present in the blood of typhoid patients? REED, however, produced focal lesions in the liver of rabbits by injecting pure cultures of typhoid bacilli into the mesenteric veins.

Apparently no investigator has yet tested the effect of typho-toxin alone, though WELSH and FLEXNER have produced circumscribed necrosis in the liver of guinea

pigs with toxalbumins of diphtheria. FLEXNER's experiments with ricin have already been mentioned.

Focal necroses do not occur in every fatal case of typhoid. According to OSLER they apparently produce no symptoms. Their presence is probably an indication of the virulence of the infective process, and one would expect to find the lesions most frequently in those who died from toxæmia and not from perforation and hæmorrhage, which are often accidents. LONGRIDGE recently reported a case of marked focal necrosis in a patient dying in the second week of typhoid, and the case described here died earlier still. Again on examination of the liver of two patients who died in the fourth week of typhoid, from hæmorrhage and perforation respectively, I was able to find only slight focal necrosis in the one and none in the other; in both the periportal connective tissue was infiltrated with lymphocytes.

LONGRIDGE suggests that if the typho-toxins were exceptionally virulent, the necrotic areas would coalesce and produce a condition hardly distinguishable from acute yellow atrophy, and also points out that in three out of two thousand Munich autopsies of typhoid, acute yellow atrophy of the liver was found.

The ultimate fate of the areas of focal necrosis is doubtful. WAGNER noted that they had completely disappeared in two and a half months after recovery from typhoid. REED, on the other hand, believed that he found evidence of previous focal necrosis in the presence of peculiar ovoid areas of dense connective tissue in the liver of a woman who died twenty-five years after an attack of typhoid.

This is a point of some interest in connexion with the views of many French pathologists as to the relation of cirrhotoses of the liver to changes in them, due to acute infective processes.

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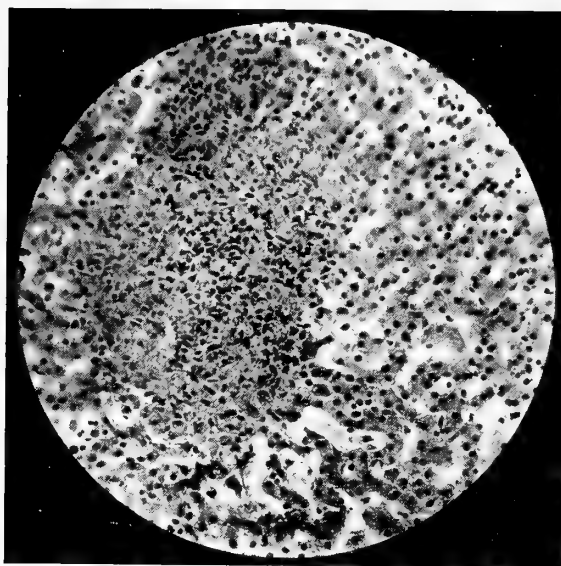


FIG. 1.—Small focus of necrosis in liver in typhoid
(to illustrate Dr. Glynn's paper)



FIG. 2.—Photograph of heart and aorta, showing multiple aneurisms
(to illustrate Dr. Hill Abram's and Dr. Lyn Dimond's paper)

MULTIPLE ANEURISMS OF THE AORTA

MULTIPLE ANEURISMS OF THE AORTA

BY

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The case is one of extreme interest, and warrants a short description of the clinical features.

Jason Rowe, aged 40, was in Royal Infirmary, January 17-April 7, 1900.

„ „ January 14-May, 1901.

„ Southern Hospital, Aug. 21-Sept. 2, 1901.

The first symptoms complained of were pains in the belly and flatulence, of three month's duration. Obvious signs of an abdominal aneurism were present, with some slight evidence of aneurism of the arch of the aorta. The apex beat was four-and-a-quarter inches from the median line. Under treatment he improved greatly, the pulsation practically disappearing. He returned to work, reappearing after an interval of some eight months, with a well-marked 'pressure' aneurism in the thorax. The apex beat was now five-and-a-half inches from the median line. The right radial, axillary, and both carotid pulses could hardly be detected. There was also marked dyspnoea.

In December, 1900, he stated he had pains in the lumbar region, and on one occasion fell when returning from work, 'his legs giving way.'

At no time was there any paralysis of the vocal cords, but there was some evidence of pressure on the right bronchus.

EXTRACT FROM POST-MORTEM REPORT

Pericardium. Contains a small quantity of straw-coloured fluid.

Mediastinum. Left bronchus is somewhat stenosed, due to pressure of the aneurism, superior mediastinum is almost completely filled up by aneurism.

Heart and Aneurism (Plate IV, Fig. 2). Hypertrophy, some dilation of left ventricle, mitral valves competent, slightly thickened by atheroma, right cavities full of *post-mortem* clot, valves normal.

Aortic semi-lunar valves—relatively incompetent as valves themselves are perfectly normal, with no thickening whatsoever. Marked universal atheroma of ascending arch of aorta, which is apparently a mere thickening of the sub-endothelial layers of the artery with no tendency to calcification or breaking down of the endothelium with consequent formation of atheromatous ulcers. At the junction of the ascending arch with transverse portion of the arch, there is a small saccular aneurism the size of a hazel nut, which is connected with the aorta by a wide mouth. The sac contains some firm decolourized laminated clot. The left carotid arises just within the orifice of the main aneurism sac, the first portion of vessel being flattened over the sac.

The left subclavian arises from the arch just beyond the aneurism by an abnormally wide orifice. The aorta throughout is atheromatous. On posterior aspect, opposite sixth, seventh, and eighth vertebrae is an elongated sac communicating with main vessel by a small opening, the dorsal aspect of sac being formed by the vertebrae, which are eroded. The sac contains a considerable quantity of laminated clot.

On the anterior aspect of the thoracic vertebrae at the level of the fifth vertebra is a small but definite aneurism, size of large pea; on same aspect, opposite seventh vertebra, there is a slightly larger swelling. In fact, the aorta in this situation shews what one might call a crop of young aneurisms. The anterior wall of the abdominal aorta for an inch above and two-and-a-half inches below the origin of the coeliac axis presents an aneurismal dilatation the size of a hen's egg. The sac is full of firm laminated decolourized clot, which extends into the right renal artery for half an inch, the remaining portion of renal artery and branches are patent, but smaller than corresponding vessels on the left side. The coeliac axis arising from the anterior aspect of the sac presents an aneurismal dilation the size of a pigeon's egg from the extreme lower end of which arise its branches. The branches of the coeliac axis show no trace of aneurismal swelling.

From the lower and anterior aspect of sac the superior mesenteric artery arises. In the first one-and-three-quarter inch it shews a pyriform dilatation, one-half inch below this dilatation and on its left border there is a small ovoid swelling communicating with the lumen of the vessel by a wide opening. Both sacs contain a large quantity of firm decolourized clot.

On the posterior aspect of the aortic aneurism on its left side there is a recent aneurismal swelling one-and-a-half inch in diameter, which is eroding the first lumbar vertebra, this sac contains ordinary *post-mortem* clot, and there are two lateral dilatations (right) of main aneurism which contains firm laminated clot.

The remaining portion of abdominal aorta shews a few atheromatous patches, but the wall of the vessel is singularly healthy, the endothelial coat seeming to be intact everywhere. The remaining portions of common iliac arteries and aorta are practically normal. Peripheral arteries slightly thickened.

Spleen : Very soft and friable, no signs of infarction.

Liver : Slightly congested, fatty, not definitely nutmeg, no infarcts.

Right Kidney : Small, renal artery is much smaller than left artery for reason given above. Capsule strips easily, cyanotic, several small scars here and there, apparently fairly recent embolic infarctions.

Left Kidney : Compensatory hypertrophy, normal, no infarcts.

A tabular form will best emphasize the features of this almost unique case :—

1. *Symptoms*. The site of the thoracic aneurisms and the relationship of the left common carotid artery to the sac, account for the deficient pulsation noted in the vessels of the right arm and the neck. During life the vocal cords were not paralysed and *post-mortem*, the evidence of pressure on the bronchi was but slight. We suggest that the dyspnoea was due possibly to spasm in the larynx, and pressure on the bronchi whilst the aneurism was pulsating. The explanation of the pains in the back is obvious.

We should like to draw attention to the history of the 'legs giving way.' One of us (A) has observed a similar condition in another case of abdominal aneurism. We suggest a temporary anaemia of the cord as the most probable explanation of this phenomenon.

2. *Number of Aneurisms*. Several cases of multiple aneurisms are recorded ; MANCE's with thirty, and PELLETIER's with sixty-three, being the most remarkable. In both these cases, however, the aneurisms were on the peripheral vessels.

According to LEBERT multiple aneurisms occur in about one-sixth of the cases. Our case shows more aneurisms on the aorta than any we can find recorded, JONAS' case coming nearest with nine.

We note also that the vessels affected correspond with LOBSTEIN and ROKITANSKI's order rather than with HUCHARD's, in that the branches of the abdominal aorta are involved, whilst the peripheral vessels have escaped.

3. *Causation*. The patient was a grain porter, age 40. He admitted alcoholism but denied syphilis. He had several healthy children, and showed no signs of specific disease. His father died from ruptured blood vessel, the mother from phthisis.

Two accepted causes of aneurism or rather of the pulmonary aortitis were present : alcoholism and heavy work.

Syphilis although usually present in multiple cases, and also in those below 40 years, may we think be excluded.

Finally in cases such as this one we have the basis of the so-called aneurismal diathesis.

We are indebted to Dr. CATON and Dr. CARTER for permission to report this case.

PRELIMINARY NOTE UPON A TRYPANOSOME
OCCURRING IN THE BLOOD OF MAN

PRELIMINARY NOTE UPON A TRYPANOSOME OCCURRING IN THE BLOOD OF MAN

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HISTORY OF CASE

The patient is an Englishman, forty-two years of age, who, for the past six years has been in Government employ with intervals of leave, as master to the Government boat, plying weekly up the Gambia river.

His illness dates back to May last year, when he broke down, after very heavy duty which often necessitated his remaining at times on watch for the twenty-four hours. Up to this time he had enjoyed good health, except for occasional attacks of malarial fever.

On the 10th of May, 1901, he was admitted into the hospital at Bathurst, with fever, under the care of the Colonial Surgeon, Dr. R. M. FORDE, to whose kindness I am indebted for permission to reproduce the temperature chart (Chart No. 1), and who will publish, at an early date, some account of the symptoms observed at this time.

On admission to hospital, patient's blood was examined (fresh preparations). No malaria parasites were seen, but Dr. FORDE informed me that he saw very many actively moving worm-like bodies, whose nature he was unable to ascertain, and it was on account of this observation that he asked me to examine the blood when the patient again returned to Gambia.

On June 1, after three weeks in hospital, the patient was invalided home, and arrived in Liverpool on June 16, in a very weak state.

On August 12, he was admitted into the Southern Hospital, under the care of Dr. MACALISTER, to whom I am indebted for the following notes. At this time his chief complaint was general weakness and lack of energy.

On admission, temperature was subnormal; no pain, but slight headache a few days previously. Tongue furred, appetite fair, no vomiting, bowels somewhat constipated; liver slightly enlarged, but no tenderness; spleen, normal in size, could be felt below the ribs on the 13th; considerable tenderness over the splenic area.

Pulse: On admission at 12-30 p.m., pulse, 120; in the evening, 92, regular in time and force, low tension, fair volume.

Heart sounds : weak and distant, otherwise normal.

Respiratory System : on admission 32, later in the day fell to 20 per minute, slight dyspnoea on exertion.

Lungs : normal.

Nervous System : no headache, legs weak ; patient said, they were much thinner than formerly, knee jerk and plantar reflexes present and easily obtainable. Sensation, normal.

Urine : normal in quantity, sp. g. 1032 ; no chlorides, no albumen.

Patient remained in hospital some fourteen days. Spleen became very painful about the 19th, but ultimately improved.

The pulse and respiration were always frequent and varied on exertion and with the temperature. The temperature was a peculiar feature as seen in chart 2. There were three short periods of pyrexia, temperature reaching from 101° to 102° in a few hours and rapidly falling to below normal, with intervals of about three days of apyrexia. On two occasions I examined the blood for malaria parasites, once in an apyretic interval, and once during a period of pyrexia ; the examinations proved negative. It will be noticed that the pulse was much more frequent during the time he was in hospital at home, very rarely being recorded below ninety beats per minute, although temperature was, as a rule, below normal. This differs somewhat from the recorded rate during the period in hospital at Bathurst.

Patient left hospital improved, the pain over the spleen gone. He went away for a change, and finally returned to Bathurst in the early part of December, 1901. On the way out he was very ill with fever, which was diagnosed as pneumonia, though the doctor informed me that it was not a typical case. The sputum was never rusty, in fact, it was more of the nature of pure blood. It is unfortunate that no record of the case was kept at this time.

I saw him for a short while after he landed at Bathurst. His appearance was much changed ; he was very much thinner, and walking readily produced fatigue.

Dr. FORDE asked me to make an examination of the blood ; unfortunately, I had arranged to go up the river, so it was not until my return on December 15 that I was able to do so. At 5 p.m., on this day, I took three drops of blood (three-quarter inch cover slips) fresh preparation.

Examination with Zeiss A lens revealed nothing ; with a higher power (Zeiss D lens) I observed the trypanosome—to be described later. Only three of these organisms were present in the three slides.

CONDITION OF THE PATIENT ON HIS RETURN TO THE GAMBIA

On his return to Bathurst, the patient was placed on the sick list as he had not yet completely recovered from the attack of pneumonia contracted on his way out.

General Symptoms. On examination of the patient with Dr. FORDE, December 18, 1901, we found his temperature 100·4, pulse 96, respiration 34.

Chief symptoms were weakness, marked loss of weight, could not walk far without feeling very tired. Patient did not complain of any definite symptom. There was no pain nor headache, but a little loss of appetite and sleeplessness at times. On December 16 he had a slight bleeding from the nose. There was no cough, but some dyspnoea on exertion. The general facial aspect which had been remarked upon by his friends was very striking. The face was puffy and congested. The eyes were sunken, the conjunctivae had a watery appearance, but were not congested; the most prominent feature was the puffiness of the lower lids, which were distinctly oedematous. On examining the body generally one noticed that the skin appeared cyanotic, especially on the chest and thighs, pressure made with the hand caused a white mark which took some little time to disappear. There was some puffiness around the ankles, the skin pitting slightly on pressure; the skin was dry; no jaundice.

Respiratory System. No cough, no expectoration. Respirations were increased in frequency. This frequency of the rhythm was very noticeable on the slightest exertion or excitement. During the time in which I observed him, his respirations were never below twenty per minute—the usual being from twenty-five to thirty, they were never laboured. Chest somewhat barrel-shaped, breath sounds normal, no dulness, slight emphysema, otherwise the lungs appeared healthy.

Circulatory System. Pulse frequent 96, regular in time and force; tension normal, artery normal. The pulse was always frequent, it hardly ever was recorded below 90 even when the temperature remained low.

Heart. Apex in the fourth interspace four inches from the mid line. Impulse could be distinctly seen, not diffuse; cardiac dulness commenced above at the third rib, and did not extend to the right beyond the mid sternal line; cardiac sounds normal, no adventitious sounds.

Digestive System. Appetite fair, no diarrhoea; had to take an occasional aperient, no pain on abdominal palpation.

Liver. Dulness, four-and-a-half inches in nipple line; extended just below costal margin.

Spleen. There was a slight bulging of the splenic area. Splenic dulness increased, diagonally measured seven inches. The edge of the spleen could be felt below the costal margin. There was no tenderness on palpation.

Nervous System. Nothing abnormal could be detected.

Lymphatic System. No definite enlargement of lymphatic glands.

Renal System. Urine normal in quantity, rather high coloured, sp. gr. 1020, acid, no albumen, no casts, some phosphates.

Chart No. 3 gives a record of the temperature, pulse and respirations from December 16th to January 5th. It will be seen that the temperature was very similar in character to that recorded in charts 1 and 2, namely, periods of slight pyrexia, lasting three to four days, with intervals of four or five days in which the temperature remained below normal. The temperature charts shew an irregular but distinctly *relapsing* type of fever.

From December 16 to December 18 the patient's temperature was raised, and on these days parasites were found in the blood, the greatest average number seen was fifteen under a three-quarter inch square coverglass. On December 19 the temperature fell below normal, and on this and for the next few days no parasites could be detected in the blood.

Progress of the Case. The patient during the period in which I observed him was never confined to his bed, and was able to take short walks in the afternoons; his appetite was distinctly good during the apyrexial periods, he did not complain again of pain over his spleen. On December 26 he was sent to the Cape for a change—a distance of seven miles from Bathurst, at the mouth of the river, where a good Government house facing the sea is built. Here I again had an opportunity to observe him, staying with him for two or three days. The fresh sea breezes appeared to produce some improvement. For the first few days he had a slight evening rise of temperature reaching to nearly 100° , and trypanosomes again appeared in the blood but no further symptoms presented themselves. The day before I returned to Bathurst (December 30) I made the following note.

‘Mr. X slept longer than usual this morning, on getting up the puffiness about the eyes is very marked, especially the right lower lid which pits on pressure; slight injection of the conjunctivae; complains again of feeling weak in the legs, the ankles are slightly oedematous; no oedema anywhere else.’ The blood was examined this day at 10 a.m., 12 and 4 p.m., no parasites were seen, the temperature on the previous night only rose four points above normal.

The patient remained five days longer at the Cape and then returned to Bathurst, temperature remained low. He seemed much improved, and was allowed to resume his duties. I made a blood examination on January 5, before he went up the river, a fresh preparation proved to be negative, one parasite was found in two smears of blood.

Case was treated with gradually increasing doses of arsenic and five grains of quinine daily.

The chief clinical features of the case were as follows:

1. General wasting and weakness, especially marked in the legs.
2. Irregular relapsing fever, temperature never very high and lasting one to four days, with at times, morning remissions; apyrexial periods of two to five days, when the temperature remained normal or sub-normal.

3. Oedema, more especially about the eyes.
4. Injection of the skin and sometimes conjunctivae.
5. Enlargement and tenderness of the spleen.
6. Constant frequent pulse and respirations (hurried breathing). These symptoms associated with no definite organic lesion.

THE PARASITES OBSERVED IN THE BLOOD

Although many slides were made and fresh preparations of the blood examined throughout the time the patient was under observation, no malaria parasites were discovered.

In fresh blood the parasite is a very minute worm-like body, very difficult to see with a magnification of three hundred diameters; especially is this the case when only few are present in a preparation, and the parasite is amongst a clump of red corpuscles; it glides along fairly rapidly in among the red cells, imparting very little movement to them. When the movements have slowed down it is seen that one end is drawn out into a whip-like process—the flagellum; the other end is bluntly conical; attached along the body is a flange-like process—the undulating membrane; the body itself is short and thick, and its substance granular. There is a highly refractile spot situated near the posterior end (Vacuole).

Movements: The parasite usually is seen progressing with the flagellum (anterior end) in front, but at times when an obstruction is insurmountable, it shoots backwards for a short distance with the blunted end (posterior) in front. Slow progression is brought about by wave-like motions started in the flagellum and communicated along the undulating membrane. The parasite in rapid motion moves in a screw-like manner, its body rotating around the longitudinal axis so that the undulating membrane appears as if it were spirally arranged around the organism. This appearance is seen in specimens of blood preserved in two per cent. formol in normal saline.

When movements slow down, I have observed on two or three occasions, parasites, apparently attached by their posterior end to a red corpuscle indenting its capsule by the waves sent along the undulating membrane. I have never observed the red corpuscle damaged in this way. On one occasion I observed the process of phagocytosis take place on a slide one hour after the blood was drawn; a mononuclear leucocyte had partially englobed the trypanosome, only the flagellum and a small portion of the anterior part of the body remaining free.

In fresh preparations, ringed with vaseline, the parasites appear to die in a few hours after the blood is drawn (one observation three hours). In such preparations, left over night, I was never able to find the trypanosome again in the morning. Atmospheric temperature varied from 90° in the day to 65° during the night. I was unable to obtain an exact measurement of the parasite in the fresh state.

Stained Preparations. Most of the blood films were stained by a slight modification of the method of ROMANOWSKY for chromatin ; this method brings out well the structure of the parasite.

The length of the parasite, in stained preparations, including the flagellum, varied from $18\ \mu$ to $25\ \mu$; in preparations which were taken on December 16 (first observation) the parasites appeared somewhat longer than those taken when they appeared in the blood again on December 27 ; the majority of specimens measured $22\ \mu$, the width was $2\ \mu$ to $2.8\ \mu$. This width, when compared to the other trypanosomes is distinctly greater in proportion to the length.

The flagellum stains a light crimson, and can be traced from the anterior end of the organism along the outer margin of the undulating membrane, stopping short of the refractile spot seen in fresh preparations ; it sets in small curves along the body, and there is always present a dip opposite the nucleus. The free part of the flagellum is about one third that of the total length, but it is difficult to say where the anterior part of the body ends and the flagellum begins ; one can always see a narrow streak of protoplasm, staining blue, for some distance beneath the free part of the flagellum.

The posterior end of the organism is roughly conical, in most specimens with the point of the cone cut away on the side remote from the undulating membrane ; it is very blunt.

The undulating membrane is a narrow unstained band, somewhat wrinkled, attached along one side of the animal ; in stained preparations, it sometimes takes on a faint pink colour.

The nucleus (the macro-nucleus of PLIMMER and BRADFORD) is situated a little anterior to the middle of the body, in some specimens occupying the whole width of the animal ; it is oval in shape and stains dark crimson, due to an aggregation of chromatin granules.

Generally about $2.5\ \mu$ from the posterior end is a dark purple spot, well marked, shewing no definite structure ; this is the centrosome (LAVERAN and MESNIL) or micro-nucleus of PLIMMER and BRADFORD. Anterior to it there is a large clear spot (vacuole) which does not stain ; the vacuole in all the specimens is well marked ; the flagellum appears to end at the upper edge of the vacuole. LAVERAN and MESNIL² point out the connexion of the flagellum with centrosome from observations on *T. lewisi*.

The protoplasm does not stain evenly, it takes on a basophil reaction, and in it are fine blue-stained granules situated chiefly beneath the attachment of the undulating membrane, and also around the nucleus. Plate VI shews the trypanosomes stained by ROMANOWSKY's method. The organisms 'set' in a characteristic manner on a

1. Plimmer and Bradford state, the size and length of the body of *T. Brucei* varies very much with the period of the disease. *Quart. Journ. of Microscop. Sc.*, vol. 45, pt. 3, p. 452. Feb., 1902.

2. Sur le Trypanosome des Rats (*T. lewisi* Kent). *Ann. de l'Institut Pasteur*, September 25, 1901, p. 684.

slide, viz., the body is generally bent at an angle opposite the nucleus (see Fig. 4, Plate V). I have observed this in most of the stained and in formalin preparations; whether this is a distinguishing feature or not is difficult to decide, but it is curious to note in film preparations that the body of *T. lewisi* does not bend but sets in a crescentic manner (Plate V, Fig. 1); in the case of *T. brucei*, the body makes three or four curves, (Plate V, Fig. 2). The number of trypanosomes present in fresh preparations (three-quarter inch square cover glass) is indicated in Chart III. It is to be noticed that during the apyrexial period, they were not detected in the blood. I have not observed dividing forms in any of the slides made.

A blood count was made on December 18, four hours after food—

Red corpuscles numbered 3,850,000 per cmm.

White „ „ 12,000 „

Hoemoglobin was 76 per cent. (Gower's apparatus).

A differential count of the white corpuscles was made on several occasions, when the parasites were present, and when few or none could be detected in the blood. On all occasions the counts showed an increase of lymphocytes at the expense of the polynuclear, the relation being generally about 50 per cent. of the latter to 40 per cent. of the former.

The only record of a trypanosome occurring as a human parasite is one by NEPVEU,¹ who, as a result of his researches in malaria carried out in Algeria, in the summer of 1888, describes various forms of organisms, streptococci, algae, micrococci, etc., as occurring in the blood of malaria patients, together with various forms of infusoria and sporozoa. He states at the end of his paper:—

‘Je n'ai jamais pu trouver, malgré le plus grand soin, la trypanomonde ou le trypanosome de DANILEWSKI, mais certainement des caractères assez nombreux semblent en indiquer l'existence, ou tout au moins la présence d'un hématozoaire très voisin. On rencontre, en effet dans le sang quelques éléments qui semblent représenter le stade sphérique ou la période de segmentation du trypanosome (vesicules à queue, vesicules en larmes bataviques, etc.)’

In 1898, he published an article *Sur un Trypanosome dans le sang de l'homme*² based on his previous observations in Algeria in 1888, in which he contradicts the statement given above. In one case, he found an organism with two flagella (trypanomonas of LABBE). In five others he found organisms which presented all the characters of the trypanosomes. In all cases they were associated with various parasites of malaria. The cases were chiefly pernicious forms of malaria, except in one subject, who was apparently in good health.

It is very unfortunate that no morphological details are given, and that only a few rough drawings published in his previous article are available.

1. Etude sur les Parasites du sang chez les Paludiques. *Mem. de la Société de Biologie*, 1891, T. III., p. 39-50.

2. Sur un Trypanosome dans le sang de l'homme. *Mem. de la Société de Biologie*. Séance du Dec. 24, 1898.

DISEASES PRODUCED BY TRYPANOSOMES IN ANIMALS

Up to 1901 four diseases occurring in various parts of the world were known to be produced in lower animals by the presence of trypanosomes.

'Surra' occurs in horses and mules in many parts of India and British Burmah, caused by *T. evansi* (STEEL).

'Nagana,' in Central Africa and probably other parts, attacking horses, and, to a less extent cattle, due to the *T. brucei* (PLIMMER and BRADFORD).

'Mal de Caderas' in Central South America and Brazil; the disease is similar to Surra and Nagana, and is produced by a trypanosome probably identical with that of *T. brucei*.

'Dourine' or 'Maladie du Coït' occurs in Algeria, South France, Spain, and Turkey; the pathological agent of which is the *T. equiperdum* (DOFLEIN), *Trypanosoma rougeti* (LAVERAN).

In February of this year Lieut.-Col. BRUCE¹ has reported a discovery by Dr. THEILER of a new trypanosome which is pathogenic to cattle. Horses, dogs, goats, rabbits, and guinea pigs appear immune. The trypanosome is twice the size of any of the ordinary trypanosomes, and Dr. BRUCE proposes to name it *Trypanosoma theileri*.

The clinical symptoms associated with these diseases, although very similar, have some minor differences, and this is more especially the case with regard to Dourine, which is not such a fatal malady as Surra or Nagana.

Dr. G. EVANS, in his report on SURRA,² 1880, describes this disease occurring in the horse as characterized by fever with jaundice, petechiae of mucous membranes, especially of eye and vagina, dropsy, sometimes albumen in the urine, great prostration, rapid wasting, with a specific parasite in the blood during life, but no characteristic structural organic lesions found after death.

The average duration of the disease is probably less than two months.

The first symptom noticed is that the animal is out of sorts; there is more or less thirst, appetite capricious, coat staring, occasionally stumbling before or dragging the hind legs on the ground; then fever, more or less high, with slight catarrhal symptoms; the eyes weeping, often a mucous discharge from the nose; the sub-maxillary gland may be tender and enlarged, general swelling of the legs; dropsy invades the sheath of horses and between the forelegs of mares; conjunctivae yellow, with claret coloured spots on the membrana nictitans at the inner corner of the eye; in mares, labia yellow with petechiae on the mucous membrane. With rest the fever subsides and appetite returns, especially for grass; thirst continues, the animal wastes away; death may be sudden, or end in delirium, or the animal may linger for days after it is down, taking its food well.

1. *The Lancet*, March 8, 1902, p. 664.

2. Report on Surra published by the Punjaub Government Military Depart., 1880, by G. Evans, M.D.

Veterinary Surgeon J. H. STEEL, A.V.D.,¹ in 1885, described very fully a disease occurring among the transport mules in British Burma, in which he found organisms in the blood of these animals, shown later to be identical with those found by EVANS in India. The symptoms were very similar to those described by EVANS. STEEL shewed that the fever was of a *relapsing* character. In inoculation experiments under the skin, the incubation period was five days ; the first acute febrile attack lasted three days with an interval of five days before the next, when the temperature would remain about normal, with, perhaps, slight evening rises. In these apyrexial periods the symptoms abated somewhat, and the blood was free from detectable parasites. Besides the anaemia, swellings, petechiae and ulcers, etc., associated with general wasting, he pointed out the enlargement of the spleen as a constant feature in the disease, and some enlargement of the lymphatic glands. Ulceration of the stomach was found after death with a general oedematous and congested condition of the organs and areolar tissues.

Surgeon-Major BRUCE,² in 1896, has described the symptoms of Nagana in various animals.

In the horse, the first noticeable features are that his coat stares, and there is a watery discharge from the eyes and the nose ; shortly afterwards there appears a slight swelling under the belly or a puffiness of the sheath may be noticed, and the animal falls off in condition, hind extremities tend to become swollen, at times more marked than at others, the animal becomes more emaciated ; eyes and gums are pale, and probably a slight milkiness of the cornea is observable ; no symptoms of pain ; up to the last a fairly good appetite ; animal falls down, unable to rise and dies of exhaustion. Other points recorded in his notes on the cases are the anaemia, red cells decreasing from 5,500,000 to 2,500,000 ; petechial spots on the mucous membranes, swollen glands. He describes the temperature as very irregular ; in a temperature chart given of a horse which had been taken into the ' Fly country ' and there contracted the disease, it is interesting to note the close relation between the presence and number of parasites in the blood to the rises of temperature. The disease started on October 4, temperature reaching 104·8 next day ; with numerous parasites in the blood, on October 21 and 22 temperature was normal (varied from 90° to 102°) ; on these days the blood was examined, and no parasites found. On the evening of October 23 temperature arose to 107°, and numerous parasites were present in the blood. Next day temperature dropped to 100° ; no parasites were found. On subsequent days the parasites increased from four hundred to five thousand on October 28 ; the evening previous there had been a rise of temperature to 106°. After the rise the parasites diminished slightly, with, at the same time, a slight diminution of temperature, until October 31, when

1. Report of Vet. Surgeon J. H. Steel, A.V.D., on his investigations into an obscure and fatal disease among transport mules in British Burma. 1885.

2. Tsetse-fly disease or Nagana in Zululand, Durban, 1894. Further report on tsetse-fly disease in Zululand, London, 1897

six thousand were present in the blood per c.mm. ; next day temperature reached 105.8° , with a drop to 102° on the following day, when no parasites were found. This rise and fall of temperature was again repeated with a corresponding appearance and disappearance of parasites, followed by another period in which the parasites gradually increased in the blood up to the time of death on November 6. The course of the disease presents a remarkable similarity to that recorded by EVANS and STEEL amongst the Surra cases in India and Burmah.

The symptoms presented in the South American disease, Mal de Caderas, are very similar to those of Surra and Nagana ; haematuria is a very frequent accompaniment and weakness and paralysis of the hind legs are the most pronounced features.

With regard to Dourine, or Maladie de la Coït, the symptoms presented in this disease are of a much less severe character. In the horse there is rarely fever. The disease shows itself in the horse after coitus in ten to twenty days, and lasts four to ten months, with swelling of the genital organs. Other symptoms present are progressive emaciation, oedema of the abdominal regions, swelling of the pastern, and weakness of the hind legs. Towards the end, eye troubles set in, paraplegia and a cutaneous eruption have been observed.

The parasites are always rare in the blood, occurring for the most part in the sero-sanguinolent fluid of the local oedemas under the cutaneous plaques, and on the mucous membranes of the vagina and urethra.

LAVERAN and MESNIL¹ state that NOCARD has been able to kill horses by inoculating the trypanosome of Dourine in four, six, and eight weeks, and they show a temperature curve of Nagana and Surra.

Chart No. 4 shows the character of the temperature and the relation of parasites occurring in the blood (indicated by dotted curve) in an ass inoculated with *T. brucei* by LAVERAN and MESNIL.

If the above symptoms in animals are compared with those described as occurring in man, it will be seen that they have many points in common ; the same cachectic symptoms—loss of flesh, weakness, similar eye symptoms, oedema, etc., accompanied by an irregular *relapsing* fever, associated with the disappearance of the parasites after the pyrexial attack. Though there is as yet no experimental evidence that the symptoms described in this case result from the presence of the trypanosome ; yet here we have a case presenting peculiar clinical features which do not show much resemblance to any known disease, and along with them is a pathological agent, allied species of which cause similar symptoms in the lower animals ; for this reason I think it is justifiable to consider this case as akin to Surra or Nagana occurring in man.

1. Laveran and Mesnil, Sur le Trypanosome du Nagana ou Maladie de la Mouche Tsétsé, *Annales de l'Institut Pasteur*, Jan. 25, 1902

THE IDENTIFICATION OF THE PARASITE FOUND IN MAN

It is now known that the course of the disease produced by a trypanosome, varies in the different animals experimentally inoculated ; some animals being more refractory than others ; and the parasite also varies in its morphological character in the different animals, and with regard to the numbers occurring in the blood. LAVERAN and MESNIL¹ and others have shown that *T. lewisi* cannot be inoculated into the larger animals, dog, cat, cow, horse, mule, etc. It produces no pathological effect in rats. Divisional forms are only seen for a short time in the blood, four to eight days after inoculation (LAVERAN and MESNIL), and it can be easily distinguished from the other trypanosomes by its morphological characteristics ; thus, comparing *T. lewisi* with *T. brucei*, the former is smaller and thinner, length measuring 24-25 μ , breadth 1.5 μ , while the latter measures 26-27 μ length, 1.5 to 2.5 μ breadth in the rat. The general aspect of the parasite is finer, the posterior end is pointed, while in *T. brucei* it is blunt. The macro-nucleus is situated at the anterior end of the body, the macro-nucleus is placed transversely as a rule and is large (PLIMMER and BRADFORD), the protoplasm stains less deeply with basic dyes. In *T. brucei* the macro-nucleus is placed centrally, the macro-nucleus is small, protoplasm stains well, and in it are chromatic granules situated anterior to the nucleus (LAVERAN and MESNIL).

PLIMMER and BRADFORD² describe Amoeboid and Plasmodial modes of multiplication as well as longitudinal division in the case of *T. brucei*, while in the *T. lewisi* longitudinal division is the rule. LAVERAN and MESNIL³ shew that longitudinal division differs in the two parasites. *T. lewisi* is more resistant to cold than *T. brucei*. LAVERAN and MESNIL³ inoculated rats successfully with blood containing *T. lewisi* after being fifty-five days in the refrigerator at 5-7°, they were unsuccessful with blood containing *T. brucei* which had been kept three to five days in the refrigerator, although a few motile organisms were present.

In the case of tsetse trypanosome, BRUCE⁴ pointed out that the parasite differed in appearance in the various animals he inoculated. PLIMMER and BRADFORD,² and LAVERAN and MESNIL³ have studied the *T. Brucei* in the horse, dog, cat, rat, mouse, etc. In the rat and mouse the organisms are always numerous and steadily increase in the blood until death, which takes place in six to nine days. In the rabbit the parasites are only found at irregular intervals ; in the goat the disease is protracted (death in two months) and the organisms are not found abundantly in the blood ; the spleen is not enlarged. In the horse the parasites were longer and thinner than in any other animal. LAVERAN and MESNIL³ state that the parasite varied from 28 to

1. Laveran and Mesnil. 'Sur le Trypanosome des Rats. *Ann. de l'Institut Pasteur*, Sept. 25, 1901.

2. Plimmer and Bradford. The Trypanosoma Brucei, the organisms found in Nagana. *Quarterly Journal, Micro. Society*, Vol. 45, Part 3.

3. Laveran and Mesnil. Sur le Trypanosome der Nagana ou Maladie de la Mouche Tsetse. *Ann. de l'Institut Pasteur*, Jan. 25, 1902.

4. Bruce, *loc. cit.*

33 μ in the horse and ass; the breadth was the same. LAVERAN and MESNIL never observed the parasites in the blood after inoculating a pig with *T. brucei*, still its blood was very pathogenic to rats, mice, etc.; five to eleven days after inoculation. Animals naturally infected with *T. lewisi* succumb to inoculation with *T. brucei* in the usual time.

In this connexion, the question of the identity of Surra with Nagana is interesting. KOCH¹ has observed no morphological differences between the two parasites. The symptoms produced in animals appear to be also identical, with the exception that cattle were considered to be more refractory to Surra, but RODGERS² has lately shown that in cattle in India, as also in goats and sheep the disease follows a similar characteristic course to Nagana in Africa. He points out that cattle may succumb to Surra, while in Africa they may not infrequently recover from Nagana.

RODGERS² has also shown that Surra can be transmitted by the bites of horse flies (*Tabanus tropicus*).

With regard to Mal de Caderas, LAVERAN and MESNIL³ state that the parasite is identical with that of Nagana. It affects horses in a similar manner, but cattle appear to be absolutely refractory.

LAVERAN and MESNIL³ point out that the parasite of Dourine, can be easily distinguished from the other trypanosomes by its pathological effects on animals, and its morphological characteristics. Cattle, sheep, goats, are refractory. The disease is only transmitted during the act of coitus. The parasites are rare in the blood occurring for the most part in the oedema fluids.

From the foregoing facts, it will be seen that it is impossible to identify the typanosome in man without inoculation experiments in the lower animals. It is quite reasonable to believe that the trypanosome I have described may be a known species modified in man; but on the other hand, I would point out that there has not been a case recorded of symptoms produced by trypanosomes amongst natives or whites in the countries where these diseases occur, though they are subject to the same risk of infection; for instance, in Africa the tsetse fly bites travellers, natives, and others just as much as animals.⁴ I have not found any record of Nagana occurring in animals in the Gambia. Horses live well in Bathurst, and from places up the river have been sent down to other parts of the coast. At a place one hundred and eighty-five miles up the river, Baia, I observed donkeys in good condition; but at some places on the West Coast horses cannot be kept. Dr. CHRISTY informs me that he has seen trypanosomes in the blood of horses in Northern Nigeria; at Jebba all horses were examined for trypanosomes before being bought by the Government.

1. Reiseberichte, Berlin, 1896.

2. *Pro. Royal Society*, May 4, 1901.

3. *Loco. cit.*

4. See Bruce, *loco. cit.*

Dr. LAVERAN, who has very kindly examined some blood films taken from the patient wrote to me that if the morphological characters are alone considered he would regard my specimen as a new species ; it is smaller than *T. lewisi* (24 to 25 μ), *T. brucei*, (26 to 28 μ) and *T. equiperdum* (25 to 28 μ), and differs from *T. brucei* in length of the flagellum and by the small number of chromatin granules in the protoplasm.

At present then it is impossible to decide definitely as to the species, but if on further study it should be found to differ from the other disease-producing trypanosomes I would suggest that it be called *Trypanosoma gambiense*.

During the time I was in Bathurst I did not observe any symptoms occurring among the natives similar to those detailed above, but Dr. R. M. FORDE informed me sometime before I examined the patient that he had come across cases among the native boatmen presenting similar symptoms, oedema, etc. I examined the blood of some native sailors on the Government launch, some fourteen in all, with negative results ; all appeared healthy.*

Specimens were very kindly collected for me of the mangrove flies by Mr. BATTY, which are often very troublesome on board the launch. I also obtained a few specimens on my journey up the river. Two varieties were caught, a large one, which Mr. THEOBALD identified for me as *Tabanus dorsovitta*, WELKA. The small one turned out to be a species of tsetse, *Glossina longipalpis*, WIEDERMANN var. *Facinoides*, WESTWOOD. The patient informed me that these small mangrove flies are very troublesome on the launch during the hot months—June, July, August—and that he himself had suffered frequently from their bites.

This species of *Glossina* has been seen by Mr. AUSTEN in Sierra Leone, and specimens have been brought from Asaba on the Niger ; by Dr. CROSS it appears to have a wide distribution in West Africa.

* Since going to press I have lately examined a series of one hundred and fifteen films obtained from native children (one to fifteen years of age), which I brought home for the purpose of estimating the prevalence of malaria in the Gambia.

In one preparation of blood taken from a child three years old, I found trypanosomes present. In the smear three parasites were counted, presenting identical characteristics ; size, shape, staining reaction, and position taken up on slide ; to the parasite described occurring in the blood of the European.

Associated with the trypanosomes were a few ring forms of malaria parasites.

The child was one of a batch of fifty examined at a native village, seven miles from Bathurst, near the mouth of the river Gambia ; these children were to all appearances healthy.

DESCRIPTION OF PLATES

PLATE V

- Fig. 1.—*Trypanosoma lewisi*; stained preparation of the blood of a rat. $\times 1400$.
- Fig. 2.—*Trypanosoma brucei*; stained preparation of the blood of a mouse. $\times 1400$. The largest organism in the photograph is undergoing longitudinal division; the centrosome, nucleus, and flagellum, have almost completely split into two. Specimen kindly sent to me by Dr. LAVERAN.
- Fig. 3.—*Trypanosoma equiperdum*; stained preparation of the local oedema fluid of an infected dog. $\times 1400$. Specimen kindly sent to me by Dr. LAVERAN.
- Fig. 4.—*Trypanosoma gambiense*; stained preparation of the blood of man. $\times 1400$.

PLATE VI

Trypanosoma gambiense; drawing from a specimen of blood stained by ROMANOWSKY's method.
 $\times 2100$.

CHARTS

- 1.—Temperature chart of case whilst in hospital at Bathurst, under care of Dr. R. M. FORDE.
- 2.—Temperature chart whilst in Royal Southern Hospital, Liverpool, under care of Dr. MACALISTER.
- 3.—Temperature chart and results of blood examinations whilst under the author's observation after his return to Bathurst.
- 4.—Temperature chart of an ass inoculated with *T. brucei* (after LAVERAN and MESNIL).

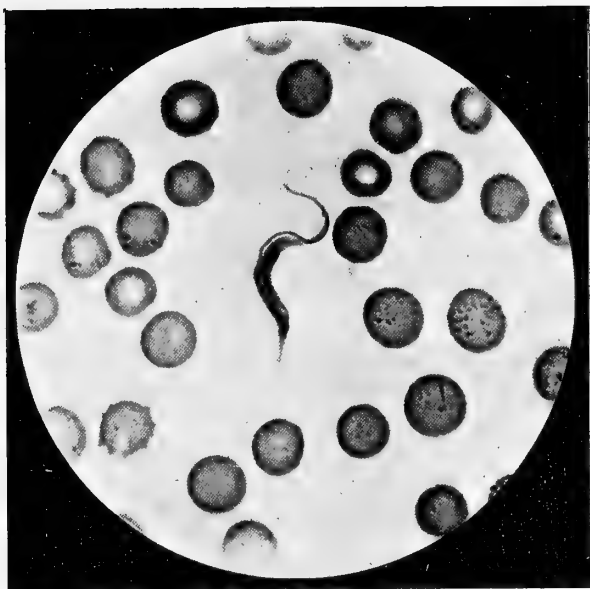


FIG. 1

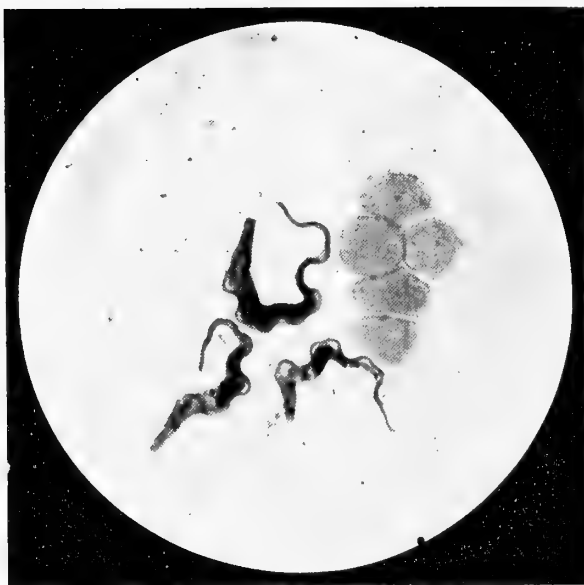


FIG. 2



FIG. 3

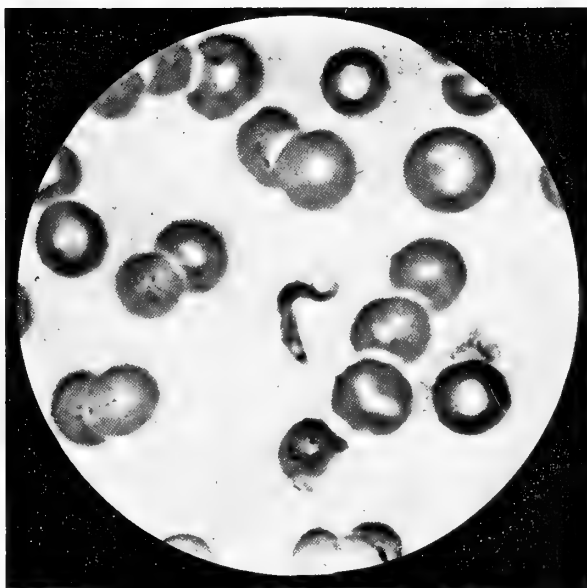


FIG. 4

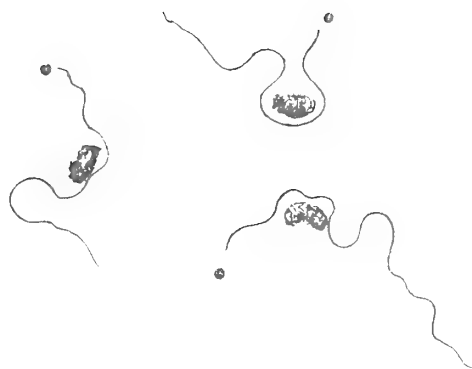
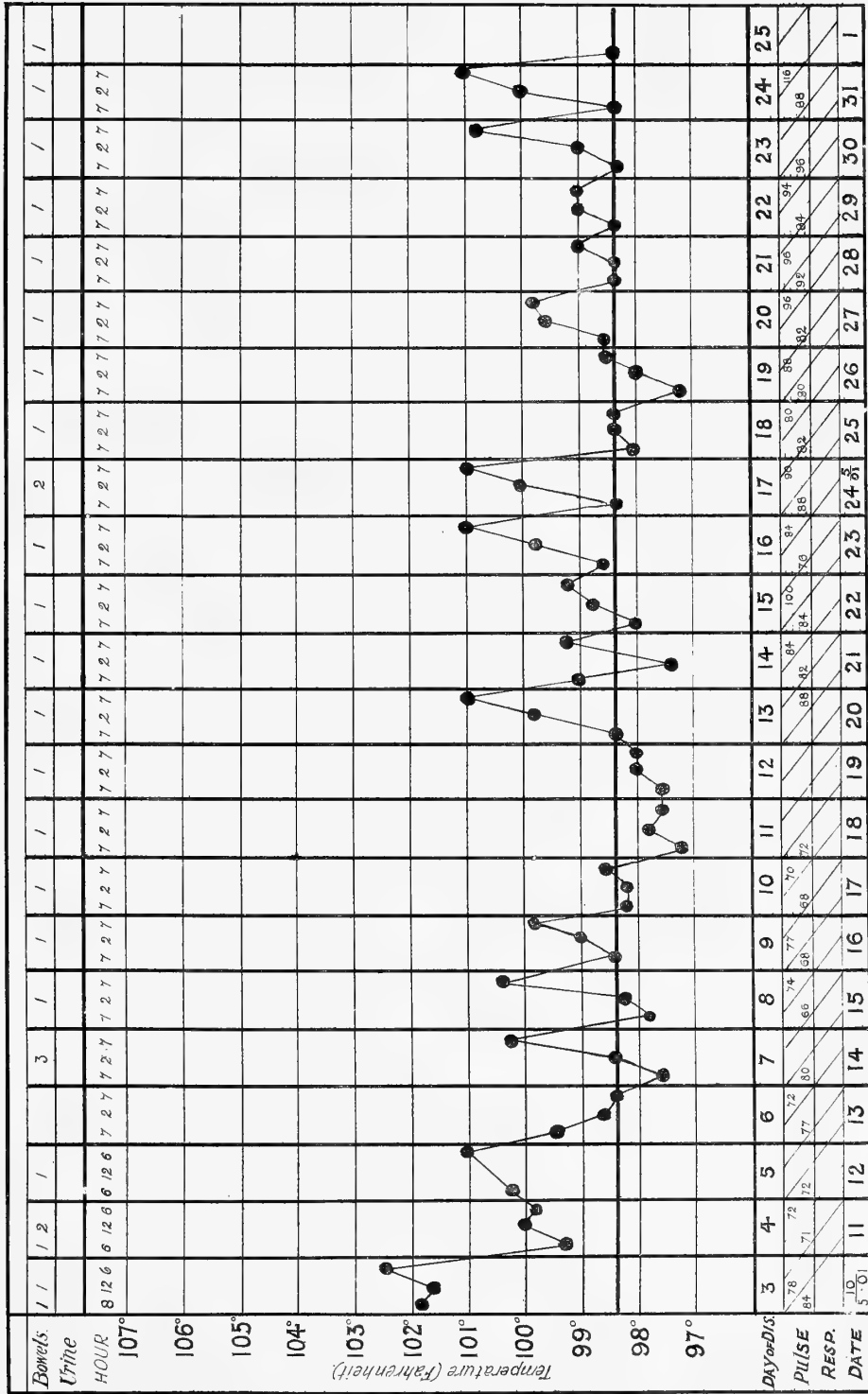


CHART N^o 1.



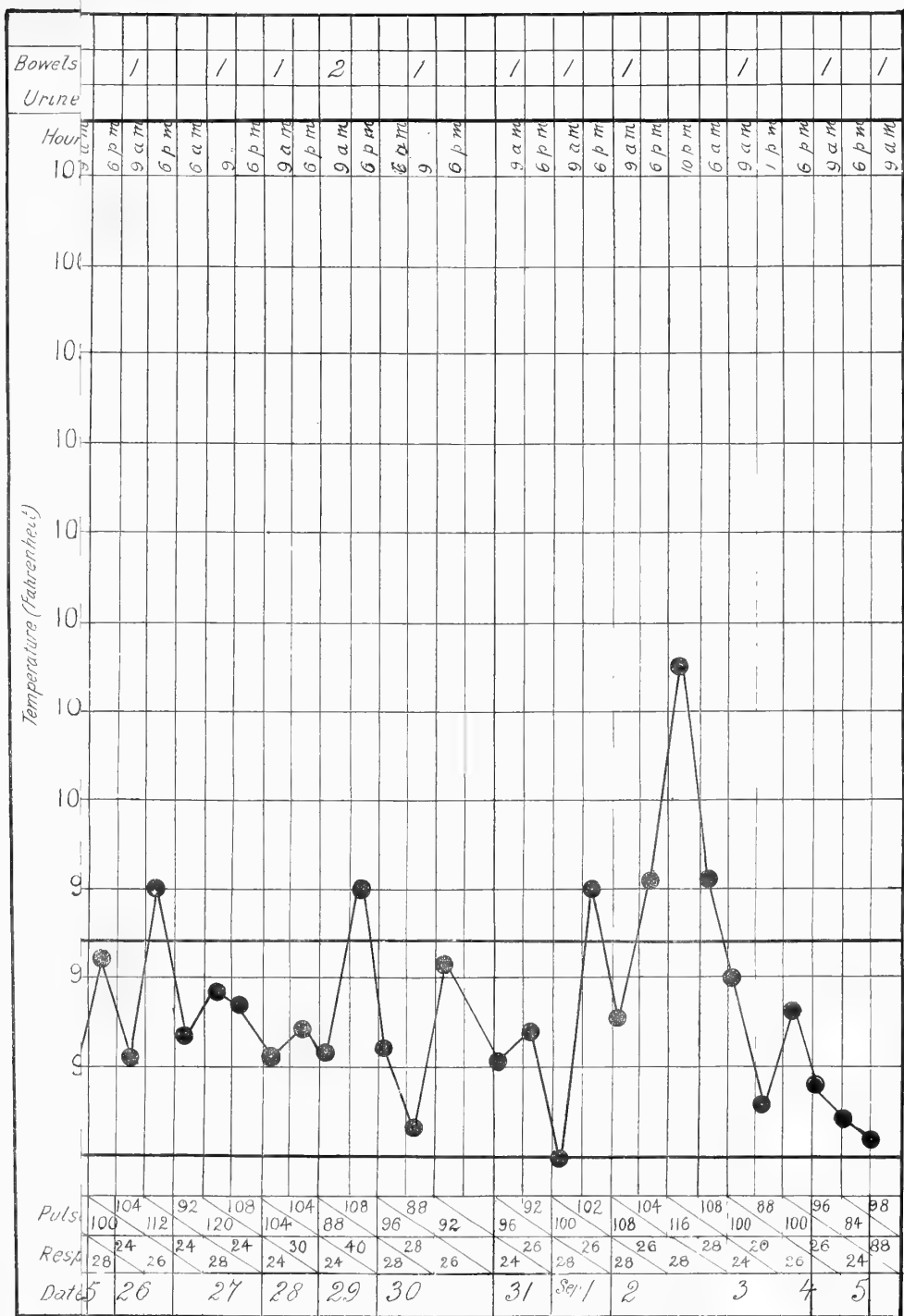
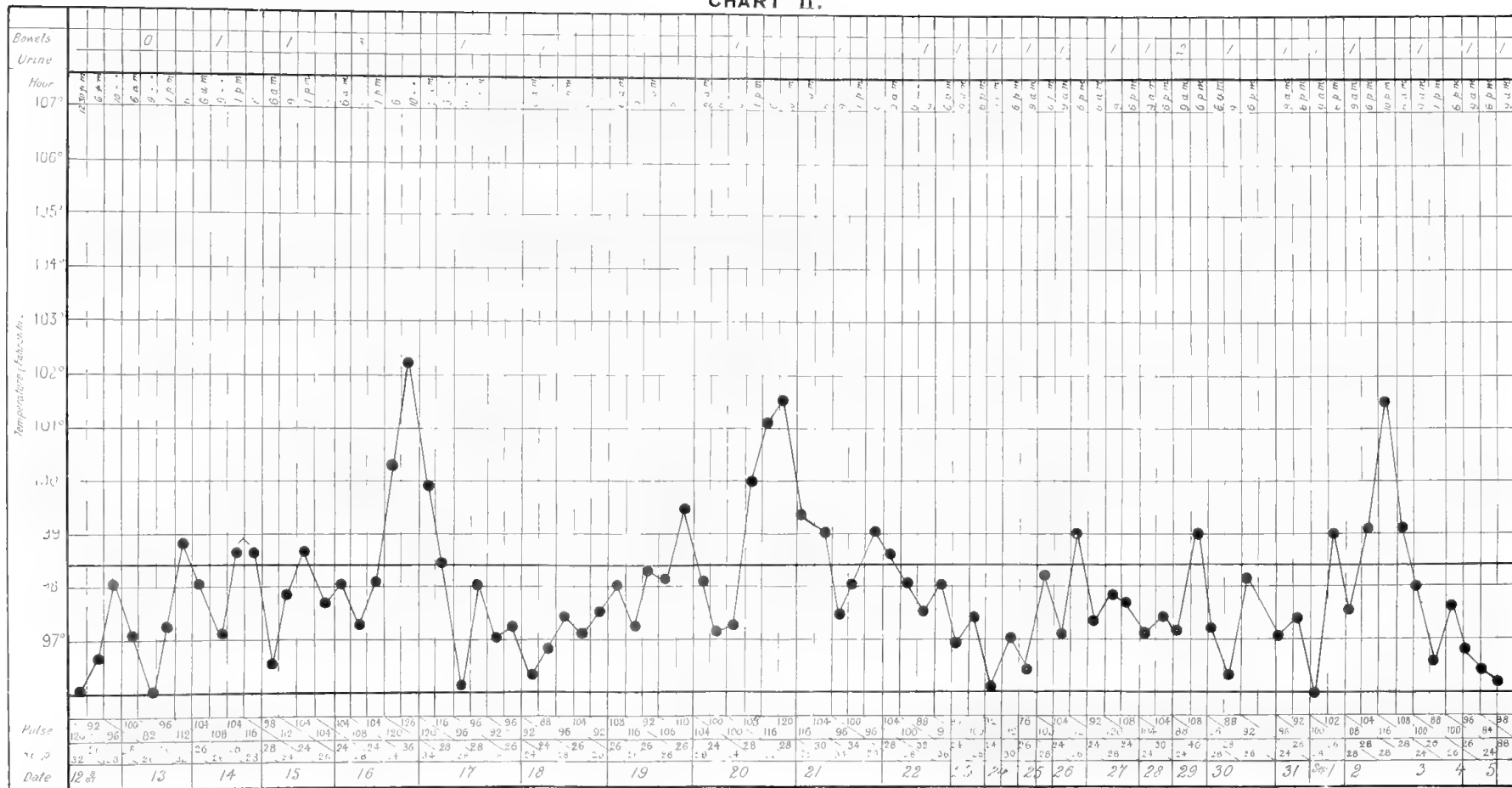
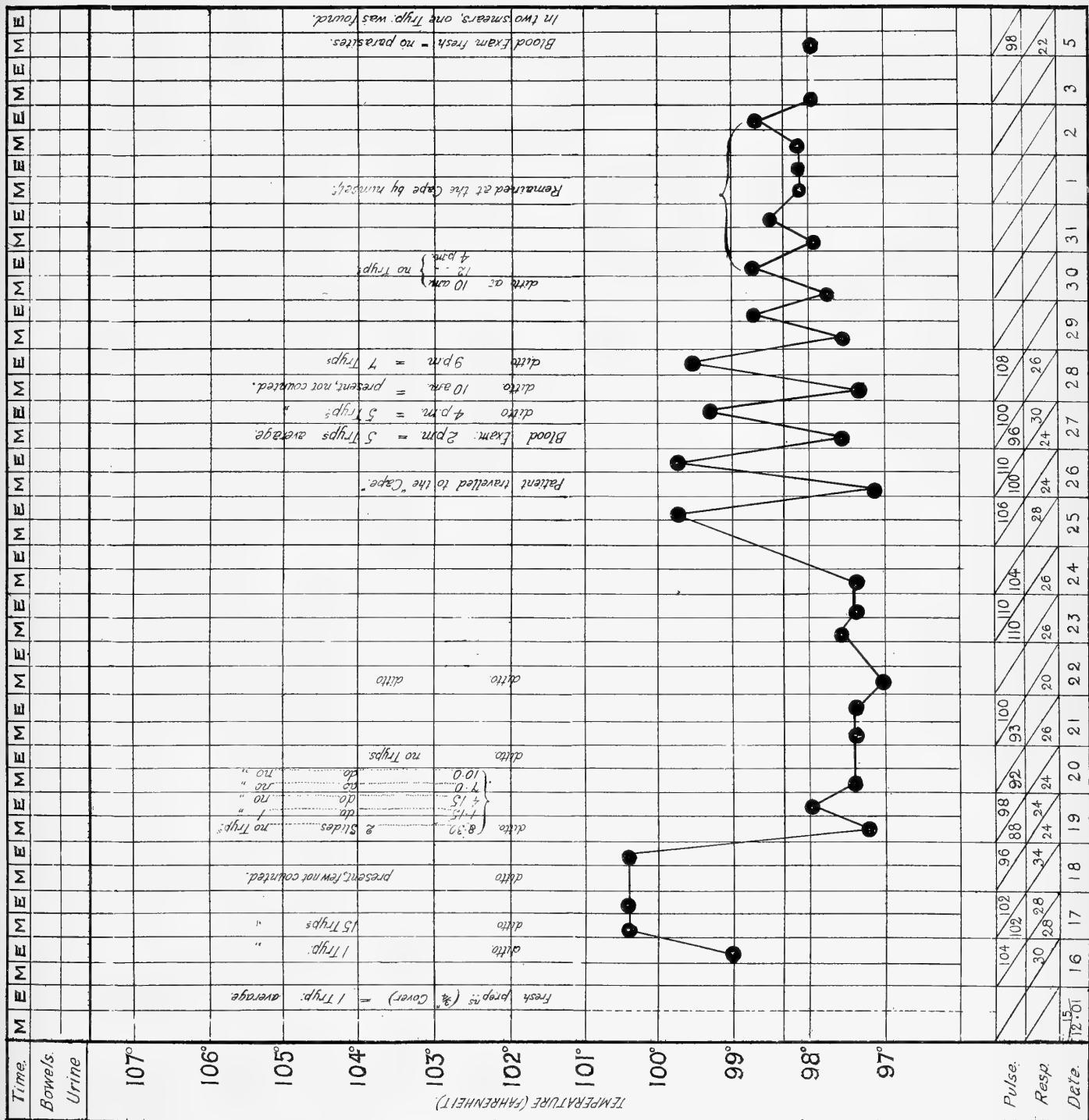


CHART II.





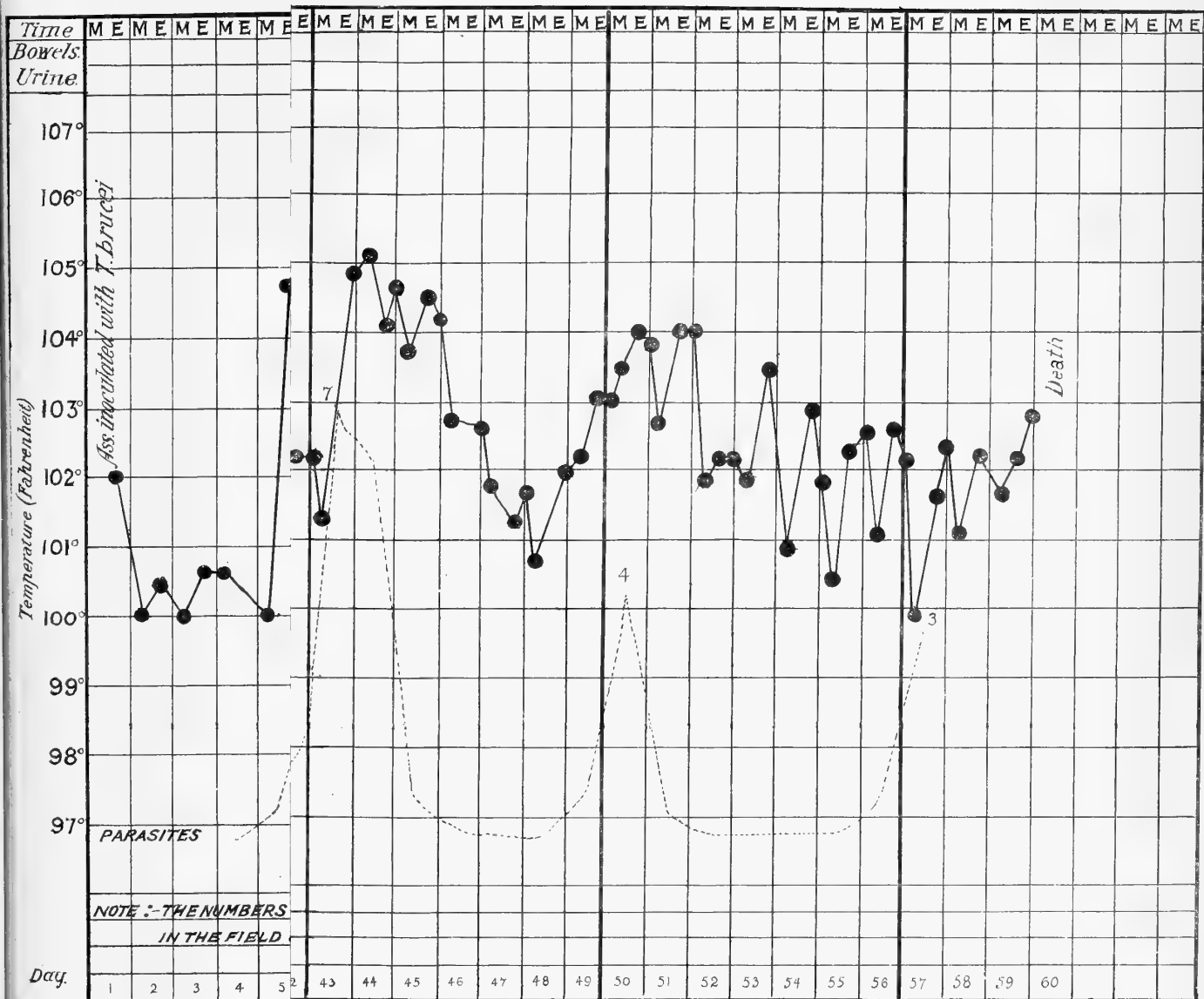
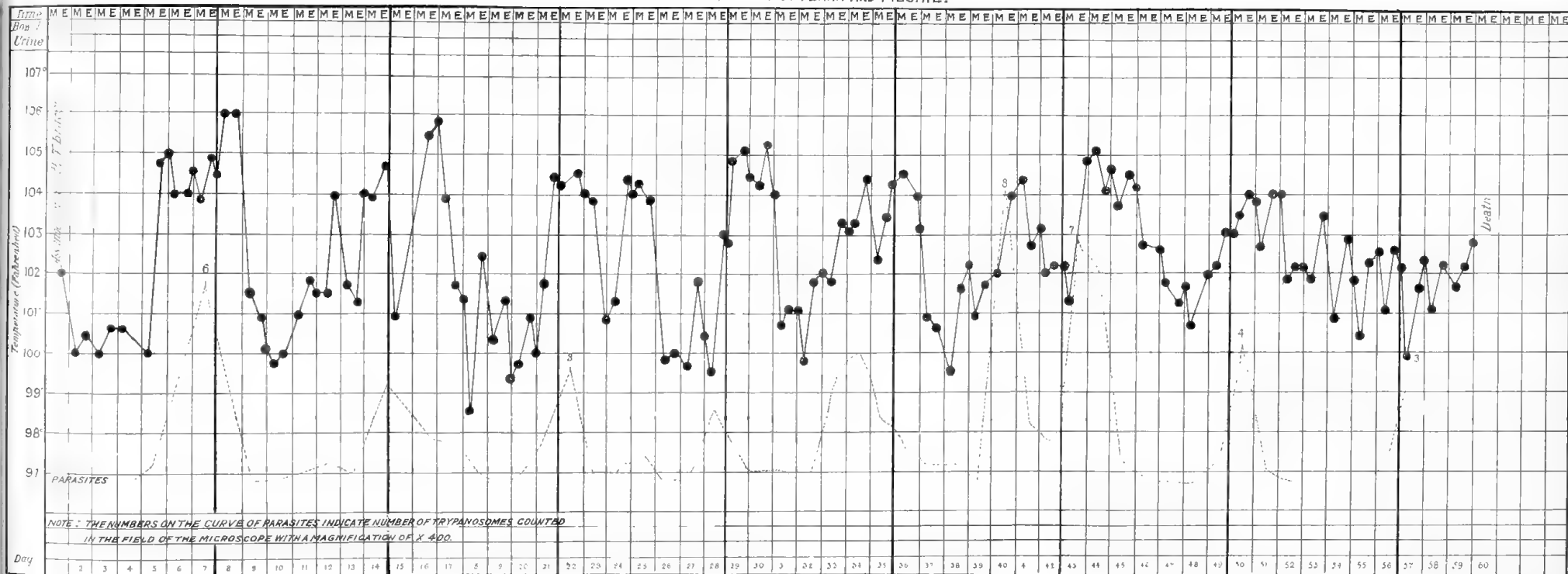


CHART N° IV, AFTER LAYERAN AND MESNIL.



QUELQUES NOTES SUR LES EMBRYONS DE
‘STRONGYLOÏDES INTESTINALIS’ ET
LEUR PÉNÉTRATION PAR
LA PEAU

QUELQUES NOTES SUR LES EMBRYONS DE 'STRONGYLOÏDES INTESTINALIS' ET LEUR PÉNÉTRATION PAR LA PEAU

PAR LE DR. PAUL VAN DURME (GHENT)

Une question des plus intéressantes en parasitologie a été soulevée récemment. Nous savons que les parasites intestinaux s'introduisent dans le corps de leur hôte par le tube intestinal lui-même, les œufs ou les embryons étant avalés avec les boissons et les aliments. C'était le seul mode de pénétration connu jusqu'au jour où les observations faites par le Dr. A. LOOSS au Caire et par le Dr. C. A. BENTLEY en Assam nous eussent fait entrevoir la possibilité d'une autre voie d'infection.

Dr. Looss¹ étudiait le développement des embryons de *Uncinaria duodenalis* (RAILLET, 1885)—*Ankylostoma duodenale* (DUBINI, 1843)—pendant leur stade de vie libre. Une goutte d'eau distillée, tenant en suspension un nombre considérable de larves, lui étant tombée accidentellement sur le dos de la main, il constata que cette application était suivie d'une vive irritation de la peau. Quelque temps après malgré que les plus grandes précautions pour éviter une infection par la bouche eussent toujours été observées, il se trouva affligé d'une sérieuse atteinte d'Uncinariose. Cette constatation lui donna l'éveil, et en Mai 1901, il publia² le résultat de recherches expérimentales. Des larves strongyloïdes furent appliquées par lui sur la jambe d'un enfant qui devait subir l'amputation du membre. Des sections de la peau, fixée une heure après, démontrèrent que les embryons s'étonent frayés une route dans l'épaisseur des tissus; le point de pénétration habituel semblait être le follicule pileux.

Dans une note parue dernièrement, Dr. BENTLEY³ communique ses observations au sujet de l'étiologie d'une affection connue en Assam sous le nom de 'Pani Ghao' ou 'Ground Itch.' Cette affection cutanée, très commune parmi les coolies employés dans les plantations de thé, est localisée exclusivement aux extrémités inférieures. Elle est caractérisée par l'apparition d'un érythème, suivi bientôt de la formation de vésico-pustules qui, dans les cas graves, peuvent se terminer en ulcérations tenaces et même en gangrène. Quoiqu'il ne puisse pas fournir une démonstration directe, l'auteur conclut de différentes expériences que les pustules sont provoquées par la

1. Dr. A. Looss, Zur Lebensgeschichte des Ankylostoma duodenale. *Centralbl. für Bakt.*, 1898, p. 441 u. 483.

2. Dr. A. Looss, Ueber das Eindringen der Ankylostomalarven in die menschliche Haut. *Centralbl. für Bakt.* 1901, p. 733.

3. Dr. C. A. Bentley, *British Medical Journal*, Jan. 25, 1902; et *Journal of Tropical Medicine*, 15th Fev., 1902.

pénétration dans la peau des larves d'ankylostome qui souvent fourmillent dans la boue des plantations. L'on sait que la grande majorité des coolies sont porteurs du parasite et que par leurs habitudes malpropres ils souillent le sol des endroits où ils travaillent.

Ces communications portent toutes deux sur le nématode *Uncinaria duodenalis*. Dans ces derniers mois, ayant eu l'occasion d'étudier un ver du même ordre, également parasite de l'intestin de l'homme :—*Strongyloides intestinalis* (GRASSI, 1883)—*Anguillula intestinalis* (BAVAY, 1877)—j'ai pu constater que ces embryons aussi possèdent la propriété de percer la peau.

Avant de fournir la démonstration de ce fait je crois utile d'exposer rapidement comment j'ai obtenu les larves au moyen desquelles j'ai fait mes expériences. Les fèces contenant les œufs de *Strongyloides* provenaient d'un Chimpanzé importé d'Africa depuis quatre mois. Soulignons ici un point digne de remarque ; les auteurs¹ qui étudièrent *Anguillula* chez l'homme trouvèrent toujours dans les selles fraîches les embryons déjà éclos ; ils attachent à ce fait une valeur diagnostique : en effet, les œufs de *Uncinaria*, qui pourraient être facilement confondus avec ceux de *Anguillula*, sont toujours évacués non mûrs. Or, chez mon chimpanzé ainsi que chez quatre autres singes infectés, je n'ai jamais trouvé d'embryons dans les excréments frais, mais uniquement des œufs non éclos à divers stades de segmentation. Et cependant tous les autres caractères : forme, dimensions, évolution étaient identiques à ceux du parasite humain. Pour étudier le développement ultérieur des œufs et des embryons, je plaçai les fèces dans une boîte de Pétri, étalées sur de la terre humide préalablement stérilisée, et les maintins à une température d'environ 25°. Les formes rhabditoïdes se développèrent rapidement en embryons mâles et femelles qui donnèrent naissance à une nouvelle génération. Celle-ci perdit bientôt son caractère rhabditoïde, chaque embryon se transformant en une larve strongyloïde. Sans insister pour le moment sur le cycle évolutif de *Strongyloides intestinalis*, disons que, à différentes reprises, dès le troisième jour nous pouvions voir quelques larves strongyloïdes. Ce qui semble confirmer les observations de GRASSI, GOLGI et MONTI qui affirment que cette forme peut provenir directement du premier embryon rhabditoïde, sans intermédiaire d'individus sexués. La larve strongyloïde est probablement la forme ultime de la vie extra-parasitaire ; à partir du 7^{ème} jour en effet on n'observe plus de transformations. Dans quelques uns de mes échantillons, particulièrement bien développés, le nombre des larves était énorme. J'eus ainsi l'occasion d'observer plusieurs fois un curieux phénomène : un matin, je trouvai, se détachant sur le fond sombre d'une de mes cultures, une trentaine de bourgeons ou brindilles blanchâtres, de 1 à 4 mm. de longueur. En les examinant avec attention on surprenait des mouvements ondulatoires et gyrotoires. Je ne puis mieux les comparer qu'à de minuscules palpes cherchant à saisir quelque chose au passage.

1. Cfr Résumé bibliographique dans, R. Strong, Infection with *Strongyloides intestinalis*. *Johns Hopkins Hospital Reports*, 1901.

Elles prenaient naissance surtout aux aspérités, s'affaissaient et disparaissaient après quelques minutes, pour reparaître un peu plus tard. L'examen microscopique nous révéla que nous avions affaire à des faisceaux compacts de larves strongyloïdes. Saisi à la pointe d'une aiguille et transporté dans une goutte d'eau, un de ces bourgeons s'évanouissait en un nuage laiteux, culture pure de larves. Le phénomène ne fit que s'accroître pendant plusieurs jours, si bien que toutes les crêtes et saillies étaient couvertes d'un rebord crêmeux. Les embryons à ce stade sont doués d'une grande activité : on les voit parcourir rapidement le champ du microscope, déplaçant les particules de toute nature qui les entourent. Leur force de résistance aux agents extérieurs est bien plus considérable que celle des embryons rhabditiformes. Alors que ceux-ci ne résistent que quelques minutes à une solution faible d'acide chlorhydrique (2%), les larves strongyloïdes y conservent leur motilité pendant une demi-journée. Portées dans du sérum sanguin elles survivent également plusieurs heures.

Tout ceci nous suggéra l'idée d'étudier l'action des embryons sur la peau. Le phénomène décrit plus haut simplifiait singulièrement la technique à suivre : nous n'avions pas en effet à isoler les larves par des manipulations compliquées, au risque d'affaiblir leur vitalité. La région abdominale d'une série de cobayes ayant été lavée et rasée, une goutte de culture pure était déposée à la surface de la peau. Pendant une dizaine de minutes on pouvait suivre à la loupe la masse grouillante se répandant dans différentes directions. Nous avions soin de maintenir la peau humide pendant environ une demi-heure. Au bout de ce temps un léger érythème était visible au point d'inoculation. En raclant l'épiderme et en examinant le produit au microscope, plus aucun embryon ne pouvait être retrouvé. Quelques heures après, la vascularisation de la peau était très apparente. Au bout de 24 heures une vésico-pustule suintante s'était formée : en même temps s'accusait une desquamation de l'épiderme qui dura pendant plusieurs jours. Nous attirons l'attention sur ce fait que la réaction ne se produit qu'à l'endroit où l'émulsion a été déposée ; le reste de la surface rasée restant intacte, les phénomènes décrits ne peuvent être mis sur le compte d'irritations mécaniques étrangères. On remarquera que les symptômes correspondent à l'affection décrite par BENTLEY sous le nom de 'Pani-Ghao.'

Des lambeaux de peau furent excisés et fixés après 30 minutes, 1 heure et 20 heures.

Après une demi-heure les larves se trouvent engagées déjà profondément dans le derme. On les rencontre le plus souvent aux alentours du follicule pileux, au niveau des glandes sébacées. Nous n'avons pu rencontrer dans ces coupes un embryon entier : mais plusieurs fois nous avons trouvé les extrémités céphalique et caudale, dont la forme caractéristique ne laisse aucun doute sur la nature des corps étrangers. Les sections ont coupé les larves transversalement, obliquement ou longitudinalement. Toutes ces figures montrent que la cuticule n'est pas parfaitement lisse ; quatre replis parallèles notamment courent le long du corps de l'embryon.

Les préparations de la peau fixée après une heure présentent des fragments de larves jusque dans le tissu aréolaire sous cutané.

Vingt heures après l'inoculation, toutes les larves n'ont pas disparu ; quoique moins nombreuses nous pouvons en voir à différents niveaux dans le derme. Ce fait toutefois n'a rien d'étonnant : les embryons parcourent une route capricieuse, reviennent sur eux-mêmes, et l'on comprend ainsi que tous n'aient pas pénétré dans la profondeur.

Nos préparations ne nous permettent pas de suivre le trajet parcouru par le ver pendant ses pérégrinations dans les tissus ; ceux-ci ne paraissent pas être notablement lésés par son passage. Looss affirme qu'il pénètre par les follicules pileux : souvent il l'y trouve à côté du poil. Jamais je n'ai trouvé une seule larve à cet endroit. Toutefois le mécanisme indiqué par Looss paraît le plus rationnel. Quoiqu'il faille admettre que les embryons se fraient activement une route dans les tissus, il est difficile de comprendre que leur armature buccale soit assez puissante pour percer la couche épidermique cornée.

Peuvent-ils par cette voie arriver jusqu'à l'intestin et s'y transformer en vers adultes ? La question est des plus intéressantes : son importance au point de vue prophylactique n'échappera à personne. J'aurais voulu compléter mes recherches à ce sujet ; mais obligé de les interrompre j'ai cru utile d'inscrire dès maintenant un second parasite sur la liste ouverte par Looss avec *Uncinaria*.

Je tiens à remercier ici le Dr. H. E. ANNETT qui n'a cessé de me prodiguer ses précieux conseils. Mes plus sincères remerciements aussi au Professeur BOYCE pour l'hospitalité qu'il m'offre dans ces annales.

EXPLICATION DES FIGURES

Fig. 1.—Dessin schématique montrant les faisceaux de larves émergeant du milieu de culture.

Fig. 2.—Un faisceau magnifié.

Fig. 3.—Extrémité cephalique d'un embryon engagé dans le derme.

Fig. 4.—Le même vu à un grossissement plus puissant.



FIG. 1

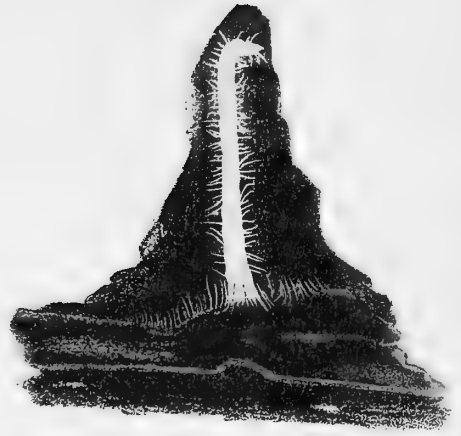


FIG. 2

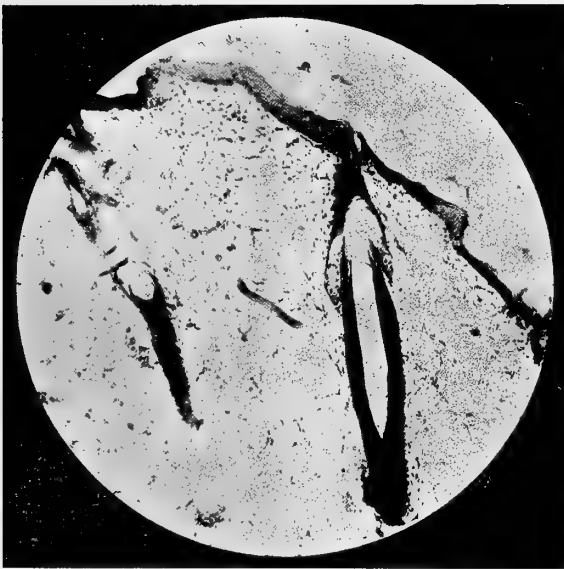


FIG. 3

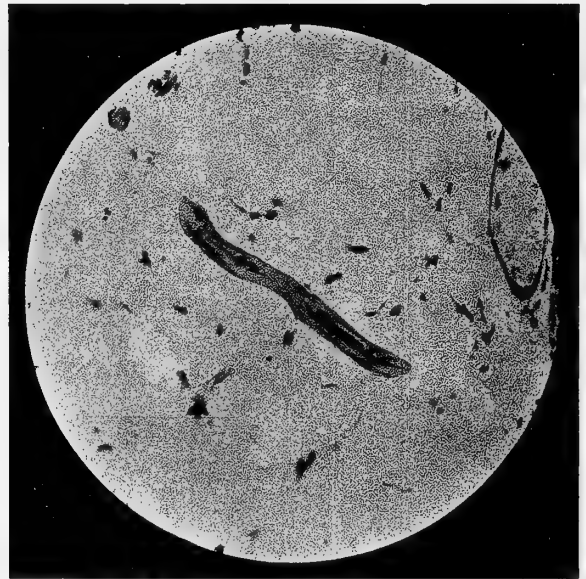


FIG. 4

REPORT OF THE
YELLOW FEVER EXPEDITION TO PARÀ

SEPTEMBER, 1901

LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR VII

REPORT
OF THE
YELLOW FEVER EXPEDITION
TO PARÀ

OF THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

BY
H. E. DURHAM

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CONTENTS

	PAGE
<i>Chapter I.</i> PREFACE	485
<i>Chapter II.</i> PRELIMINARY THOUGHTS ON THE PROBLEM OF THE ETIOLOGY OF YELLOW FEVER	487
<i>Chapter III.</i> PRELIMINARY REPORT (reprinted from <i>British Medical Journal</i>)	489
INTERIM REPORT ,, ,, ,,	492
<i>Chapter IV.</i> RECENT OBSERVATIONS ON YELLOW FEVER ETIOLOGY—	
<i>A.</i> SANARELLI'S Bacillus	494
<i>B.</i> Mosquito Transference	499
<i>Chapter V.</i> OWN OBSERVATIONS ON YELLOW FEVER ETIOLOGY .	510
<i>Chapter VI.</i> NOTES ON POINTS CONNECTED WITH YELLOW FEVER—	
<i>A.</i> 'Typical Bites'	518
<i>B.</i> Lymphatic Glands	519
<i>C.</i> Urine	522
<i>D.</i> Kidney, Spleen, etc.	524
<i>E.</i> Illness at the Instituto Lauro Sodré, and our own Infection	526
<i>F.</i> Yellow Fever on Ships	530
<i>Chapter VII.</i> NOTES ON AGUE AT PARA, etc.	534
<i>Chapter VIII.</i> NOTES ON GENERAL HEALTH AT PARA	542
<i>Chapter IX.</i> ODD NOTES. (<i>A</i>) PRICKLY HEAT. (<i>B</i>) DREPANIDIUM, etc.	559

REPORT OF AN EXPEDITION TO PARÀ, BRAZIL, TO STUDY YELLOW FEVER

PREFACE

The sad loss of my comrade, WALTER MYERS, after some nine months of constant companionship, though already engraved on the records of scientific research and adventure, cannot be passed over in silence. Shortly before we were struck, almost simultaneously, with the fever, we had agreed to confine our attention to a short programme, which would possibly have allowed us to return home some two months later. This, however, was not to be. The defects of this report will be a tribute to the lack of his friendly criticism, and the omissions would have been fewer had his energy and ability been available for continuing our observations nearer to the stage of completeness.

A more pleasurable task is to record the appreciation of, and gratitude to, those from whom we received assistance in the furthering of our aims. First and foremost is due our thanks to Dr. JOSÉ PAES DE CARVALHO, whose constant solicitude for our welfare, both in health and sickness, and whose position as the worthy Governor of the State of Parà rendered the prosecution of our researches possible.

To Dr. PONTES DE CARVALHO, Director of the Hospital Domingos Freire, which is reserved for the treatment of yellow fever patients, is due our thanks for allowing us all facilities in obtaining material from the cases under his charge. Dr. FRANCISCO MIRANDA, of the Sanitary Service, and several of the doctors in charge of the hospitals afforded us aid.

Mr. C. L. TEMPLE (Acting British Consul), Mr. BEALE (of Messrs. Singlehurst, Brocklehurst & Co.), and Captain CRIMP and Mr. COLLARD (of Messrs. Booth & Co.) also lent us their aid, often, be it said, at much personal inconvenience; we were much indebted to these gentlemen for their invaluable help.

Arriving in Parà on August 24, 1900, we were unable to commence for some weeks owing to the hebetude of the Custom House officials in the release of our apparatus, which had arrived some two weeks before us, and which eventually was liberated on September 13. We were able to obtain fifteen autopsies on yellow fever cadavers up to January 15, when work was interrupted by our own infection. After my return, on February 15, the prevalence of the fever had diminished so much that

cases which came into the fever hospital were few and far between, and only two further autopsies were obtainable. The difficulty of obtaining material, which had been our chief obstacle all through, owing to the absence of any system of control or notification and isolation, now became so great that it was not thought expedient to continue.

Having ascertained that there was no use in proceeding to Havana, it was judged best to return home; but I must here express my thanks to Surgeon-General STERNBERG and Major REED for their kind offices. I have also to thank the Sanitary Officers in Jamaica and British Guiana for kindly and courteously replying to my enquiries. Mr. THEOBALD has kindly identified the mosquitoes collected.

II. PRELIMINARY THOUGHTS ON THE PROBLEM OF THE ETIOLOGY OF YELLOW FEVER

Before setting out upon such a mission as that of the investigation of the etiology of yellow fever, it was necessary to frame a sort of programme of the possibilities and the modes to be adopted in dealing with the same.

At the outset it appeared that the true infective agent of yellow fever has as yet escaped recognition. Though yellow fever is by no means a virgin field for investigation, there has been little or no agreement between different observers, in regard to their respective claims. The writings of some authors hardly give evidence that their methods were sufficient or accurate enough to be of value.

The study of yellow fever must be begun again from the commencement, and any organism which may be found will not only have to be constantly found, but it will also have to afford an explanation why it has escaped the attention of other observers. That a yellow fever microbe exists there can be no question. Whether it be protozoal or bacterial in nature, it will be necessary to endeavour to define the mode of transmission and its mode of life in the outer world. The possibilities which may be encountered with either a protozoal or bacterial parasite, suggest a number of themes for consideration.

By prejudice there may be an inclination to feel that either kind of parasite may be involved. The epidemiology of the disease (prel. note) is suggestive of the action of an intermediary transmitting agency; this perhaps might better have been referred to as a transmitting *agent* rather than as an intermediate *host*.

In the light of our knowledge of other diseases due to animal parasites, it is highly suggestive that this form of parasite would occur; the apparent limitation of the geography of the disease is likewise suggestive, but though no lasting and endemic hold has been taken in northern climes, the inability to do so has possibly been rather overrated by writers of text-books.

On the other hand, the onset and features of the disease, its rapid course and the subsequent immunity are all more in accordance with our knowledge of bacterial diseases. While granting that we know comparatively little of diseases due to protozoal parasites, the hope would rather be for the establishment of a bacillary agent which might offer the opportunity for therapeutic treatment (as by an antitoxin); on the other hand, *a priori* none such would be likely for a protozoal disease in which treatment as yet is based on mere empiricism.

In hunting for protozoal parasites, experience with the parasite of Tsetse disease and of malarial fever should prove useful. It will be necessary to search everything in the fresh state—blood at various stages of the disease, and the organs as soon after death as possible. As with the Tsetse parasite the centrifuge may be useful in separating the protoplasmic structures in the blood from the heavier red blood corpuscles.

For stained specimens careful fixation with formaldehyde and osmic vapour must be practised, and a variety of staining methods—'Romanowsky,' haematoxylin, acidified basic stains, etc,—tried. Lastly, any odd or unfamiliar object must be sketched and its size determined. At autopsies it will be necessary to examine the probable paths of infection; that is to say, the superficial lymph glands in various regions. Should events guide satisfactorily it will be necessary to examine possible intermediary agents such as gnats.

Several considerations have to be borne in mind in enquiring into a 'new' disease from the bacterial point of view. In the first place the gross lesions, especially those of the liver in yellow fever, give no probability that the bacterium is especially localized in these parts; for instance, a very small amount of the botulismus toxin is sufficient to produce most intense fatty change in the liver, provided the animal lives long enough. This time factor is a consideration which apparently has not entered the minds of experimenters who have endeavoured to reproduce yellow fever in animals, it is unlikely that the lesions could be produced when a period of twenty-four to forty-eight hours only elapses between inoculation and death. In making experiments on animals it will be necessary to gauge the doses to allow the animal to survive several days.

It is unlikely that a highly septicaemic condition or that large numbers of bacteria become localised in the tissues (as in plague) otherwise it is difficult to understand why such bacterium has hitherto defied recognition. The two bacterial diseases which seem to be most apt for analogy, or the pathology of which might be kept to the fore, are botulismus and tetanus. In the former VAN ERMENGEN only succeeded in getting very few colonies of living bacilli from the organs of the fatal cases; but whilst he thus proved that actual infection had taken place he was able to reproduce the disease by absorption of toxin through the alimentary canal. Tetanus is also not particularly encouraging, for, with a scarcely discoverable number of individual bacilli, fearful havoc is wrought by intoxication. What has been already written in regard to paths of infection in a protozoal disease can only be reiterated for a bacillary one; it may be that in or about the site of primary infection it may be more easy to discover an organism, or, by examining the bottom of centrifugalized material. In the symptoms as described there does not appear to be much that suggests localizing—the vomiting, kidney and liver mischief might be purely toxic. On the other hand, pain in the epigastrium, upon which some authors lay much stress, is suggestive of some lesion possibly due to special localization.

It is not likely that the supposed bacterium will thrive on ordinary media, it might be, however, that insufficiently large proportions of tissue have been taken. This will apply to anaerobic as well as aerobic trials.

As general routine it will be sufficient to use liquid broth cultures in the first instance; any organisms that should grow freely must be found in pure condition (by observation and plating) ere it can be granted an etiological position.

Lastly, it must be remembered that a bacterium may produce very different effects according to the mode by which it is introduced into the system. (Compare experimental inoculation of animals with certain bacteria).

III. REPORTS

A. PRELIMINARY REPORT¹

Notwithstanding the acumen and the number of those who have attacked the question of the prevalence of yellow fever, much mystery and uncertainty still enshroud the epidemiology of the disease. The etiology, also, is not yet determined with certainty; the claim of SANARELLI that his so-called '*Bacillus icteroides*' is the true cause of yellow fever has not found favour with several workers who have made search in this direction. By the kind invitation of Dr. STERNBERG, Surgeon-General of the U.S. Army, we had the opportunity of conferring with the commission (Drs. REED, CARROLL and LAZEAR) appointed by him to study the question in Cuba. We may here express our gratitude to these gentlemen for the most kind and courteous reception that they extended towards us, as well as to Major GORGAS (U.S. Army), the head of the bureau of inspection of infectious diseases in the city of Havana. We also had the pleasure and advantage of meeting Dr. CARTER (of the U.S. Maine Hospital Service), Dr. GUITERAS (now Professor in the Havana School of Tropical Diseases), Dr. FINLAY, and Drs. BANGO and MARTINEZ (practitioners in the city).

Amongst the many conflicting opinions and statements concerning the disease it appears certain that neither the handling of or contact with yellow fever patients nor the performance of necropsies is capable *per se* of conveying the disease to non-immunes. It also appears probable that general ship's cargoes and the fomites of patients are also not directly infective; here, however, the evidence is not conclusive, and the present quarantine regulations require disinfection of all clothes and personal effects before they may be introduced into the United States.

It seems to be fairly definitely established that a yellow-fever patient may become a danger by 'infecting the house' in which he is placed. Given that a house is 'infected,' a visit by a non-immune person entails considerable risk of contracting the malady. It is alleged by some that visits made at night are more dangerous than those made during the daytime; but here the evidence is not very clear, and is more of the nature of an opinion. The nature of the essential factor present in an 'infected house' is as yet mysterious. One house after another in a street may become 'infected' without any apparent intercommunication of the inmates; the infection may skip over one or more houses and reappear at some distance. There are those who are bold enough to predict in a village that such and such a house will yield one or more cases of the fever on or about a certain day; and, naturally, they claim to be true prophets.

1. Reprinted from *British Medical Journal*.

Of the interesting and important facts which have been ascertained, those elucidated by Dr. CARTER in his study of outbreaks at Orwood, and TAYLOR (Miss) in 1898, are second to none.¹ The conditions were such that the intervals between the introduction of 'infecting' cases and the onset of secondary cases could be followed with accuracy. Dr. CARTER finds that an interval of about fourteen to twenty-one days obtains before the first secondary cases occur. The house is then in an 'infected' condition, and exposure for a few hours (for example, in one case four hours and a half) can lead to infection, with the incubation interval up to the normal four or five days. This was exemplified to us by the history of a case at Quemados, for which we are indebted to Dr. REED. In a house which had been occupied by non-immune officers all last year, two cases of yellow fever occurred this summer; one of these was unfortunately fatal. However, a male and a female nurse who had been occupied in tending the patients did not acquire the disease until about a fortnight after the death occurred. Other sources of infection could be excluded in these cases. No further cases occurred, as the house was cleared and liberally treated with perchloride. The slight epidemic, however, spread to other houses down the street, although they were detached and surrounded by a small amount of garden space.

This curious and somewhat prolonged interval is suggestive of a development of the infecting factor in or about some agent or matter in the domicile. What may be the nature of this supposed agent is not yet demonstrated, but the suggestion propounded by Dr. C. FINLAY, of Havana, some twenty years ago, that the disease was spread by means of mosquitoes hardly appears so fanciful in the light of recent discoveries in ague convection as appeared in the days when the idea was first broached. Dr. FINLAY's hypothesis is able to account for several curious points which obtain with yellow fever. Thus the limitation of the disease to the 'yellow fever zone,' where frost is unknown, the coincidence of yellow fever and rainy seasons, the cessation of the disease when the temperature falls below a certain point, and its non-recrudescence in an infected locality after a frost, are all compatible with an agency, such as a gnat, which becomes too sluggish to bite, or indeed which dies out in unfavourable climatic conditions. Such a theory also explains the curious spread of the disease from house to house, which has already been referred to. Another point is that the sanitary condition of a house may be good, and yet it may be severely 'infected.' An example of this was shown by the case of one of the leading hotels in Havana, of good sanitary repute, but the source of many fever cases this summer. The above sketch will suffice to show that some means of transmission by the aid of an intermediate host—a town-loving host for this town-loving disease—is to some extent more plausible than might be anticipated. Whether that hypothetical host is of the nature of a gnat remains unknown.

1. *New Orleans Medical and Surgical Journal*, May, 1900

It is commonly stated that one attack of the fever confers a long and lasting immunity against further attacks. The completeness of this immunity, however, is called in question by the cases which we had the opportunity of seeing. Thus out of the two or three dozen cases we saw, no fewer than four were reputed to have had previous attacks; the previous attacks were not believed in as genuine yellow fever by those physicians who accept a rigid immunity as the fact. A few words may be said of one of these cases as illustrative of several points:—

‘A lady had an attack of yellow fever in New Orleans, at the age of nineteen, in 1866; her account of the symptoms she experienced, and the fact that her sister had a “typical” attack at the same time tend to the conclusion that the illness was indeed yellow fever. Last year she was occupied as an “immune” in nursing yellow fever cases in Cuba during July and August, and then remained in the outskirts of Havana (Cerro) for the winter and spring. This summer she went to live in an insanitary house close to the hotel we have already alluded to as an infected house, situated in an “infected” neighbourhood. She soon contracted an illness of the nature of which there could be no doubt—a severe and prolonged attack of yellow fever.’

But our knowledge of infectious diseases all goes to show that a complete and absolute immunity is never acquired by a single attack, though second attacks are usually of a comparatively mild, or at any rate recoverable, type.

The coloured people and natives are also supposed to escape the disease, but we are informed that this is a statement which is not true, although so frequently repeated in text-books. We were lucky enough to see one negro during the course of a typical attack. The Cubans and the Cuban doctors are in the habit of asserting that the Cuban system is not capable of having yellow fever. It appears, however, that they are not unknown to suffer from a disease called ‘borras.’ Clinically ‘borras’ is like yellow fever—sometimes with black vomit and death with suppression of urine; pathologically the lesions of yellow fever are said to be present in fatal cases. The Cubans also suffer and die from ‘pernicious malarial fever,’ the symptomatology and pathological anatomy of which are very suggestive of yellow fever. We were informed that the Cuban children frequently suffer from mild attacks of fever, which our informants believe are really mild attacks of yellow fever, and which give a comparative immunity in after-life.

It has already been mentioned that a sojourn of a non-immune for some hours in an infected house is very likely to lead to an attack; and that this is supposed to be especially the case at night. Since many of the cases we saw were soldiers, their movements could be traced to some extent, and in more than one instance there was evidence of a single exposure after breaking bounds for a single night. Similar experiences are told of ship’s crews under similar circumstances; another example is to be found in the case of one hundred American military prisoners who were confined

in a barrack in which there had been several hundred deaths through yellow fever during the previous year of Spanish occupation ; none of these prisoners acquired the disease, but some twenty-five of their custodians fell ill or died of the fever.

Through the courtesy of Major GORGAS we were enabled to see the distribution of the fatal cases of yellow fever in the city for the past ten years. In these it was rather striking that the more disreputable quarters of the town were free from deaths from this cause. Immigrant women are few, and presumably the inhabitants are all 'immunes ;' still it is not improbable that the infective agent is harboured amongst them.

These few notes on some epidemiological points are submitted in the hope that they may be of some interest to students of the perplexing questions and of the unravelled natural history of yellow fever.

B. ABSTRACT OF INTERIM REPORT¹

1. Sufficient search reveals the presence of a fine, small bacillus in the organs of all fatal cases of yellow fever. We have found it in each of the fourteen cadavers examined for the purpose. In diameter the bacillus somewhat recalls that of the Influenza bacillus ; as seen in the tissues it is about $4\ \mu$ in length.

2. This bacillus has been found in kidney, in spleen, in mesenteric, portal, and axillary² lymphatic glands, etc., taken from yellow fever cadavers directly after death. In the contents of the lower intestine apparently the same bacillus is found, often in extraordinary preponderance over other micro-organisms. Preparations of the pieces of 'mucus,' which are usually if not always present in yellow fever stools, at times may almost present the appearance of 'pure culture.'

3. Preparations of the organs usually fail to show the presence of any other bacteria, whose absence is confirmed by the usual sterility of cultivation experiments.

4. It is probable that this same bacillus has been met with, but not recognized, by three other observers. Dr. STERNBERG³ has mentioned it ; and he has also recorded the finding of similar organisms in material derived from Drs. DOMINGOS FREIRE and CARMONA Y VALLE, but he did not recognize its presence frequently, probably on account of the employment of insufficiently stringent staining technique.

5. It is probable that recognition has not been previously accorded to this bacillus by reason of the difficulty with which it takes up stains (especially methylene blue), and by reason of the difficulty of establishing growths on artificial media.

1. The completion of the interim report of which this is an abstract was interrupted by the onset of attacks of yellow fever in both of us. The loss of my much lamented colleague renders it advisable to submit this shortened report only for the time being.—H.E.D.

2. We find these constantly enlarged and much injected, though whether this is specific we are not able to say

3. Report on Etiology and Prevention of Yellow Fever, 1890.

6. The most successful staining reagent is carbol fuchsin solution (ZIEHL), diluted with 5 per cent. phenol solution (to prevent accidental contamination during the long staining period) ; immersion for several hours, followed by differentiation in weak acetic acid. Two hours staining period may fail to reveal bacilli, which appear after twelve to eighteen hours. The bacilli in the stools are often of greater length than those in the tissues, and they may stain rather more easily ; naturally the same is true of cultures.

7. Since the bacilli are small and comparatively few in numbers, they are difficult to find. To facilitate matters at our last two autopsies (14th and 15th), a method of sedimentation has been adopted. A considerable quantity of organ juice is emulsified with antiseptic solutions, minute precautions against contamination, and for control being taken ; the emulsion is shaken from time to time and allowed to settle. The method is successful and may form a ready means of preserving bacteria—containing material for future study. The best fluid for the purpose has yet to be worked out ; hitherto normal saline with about one-fifth per cent. sublimate has been employed.

8. Pure growths of these bacilli are not obtained in ordinary aerobic and anaerobic culture tubes.

9. Some pure cultures have been obtained by placing whole mesenteric glands (cut out by means of the thermocautery) into broth under strict hydrogen atmosphere. Investigation into the necessary constitution of culture media for successful cultivation is in progress.

10. Much search was made for parasites of the nature of protozoa. We conclude that yellow fever is not due to this class of parasite. Our examinations were made on very fresh organ-juices, blood, etc., taken at various stages of the disease, with and without centrifugalizations,¹ and on specimens fixed and stained in appropriate ways. We may add that we have sometimes examined the organs in the fresh state under the microscope within half an hour of death.

11. The endeavour to prove a *man-to-man* transference of yellow fever by means of a particular kind of gnat by the recent American Commission, is hardly intelligible for a bacillary disease. Moreover, it does not seem to be borne out by their experiments, nor does it appear to satisfy certain epidemiological conditions. It is proposed to deal more fully with the endemology and epidemiology of the disease on a later occasion.

12. We think that the evidence in favour of the etiological importance of the fine small bacillus is stronger than any that has yet been adduced for any other pretended 'yellow fever germ.' At the same time there is much further work to be done ere its final establishment can be claimed. The acquisition of a new intestinal bacterium would explain the immunity of the 'acclimatized.'

1. We have found this sometimes useful in examining the blood of ague patients.

IV. RECENT OBSERVATIONS ON YELLOW FEVER ETIOLOGY

A. SANARELLI'S BACILLUS

SANARELLI¹ claimed from the examination of twelve cases of yellow fever that he had established the etiology of the disease. He claims to have found his *B. icteroides* seven times, that is to say, in more than half of the cases. An examination of his account shews that much contamination with a variety of bacteria was met with, in fact, it was only from two cases that pure results were obtained, whilst in five cases it was not met with at all. The cases may be summarized shortly :—

Case 1	Autopsy	18 hrs. after death	Variety of bacteria; no <i>B. icteroides</i>	. = 0
„ 2	„	2 „	Pure <i>B. icteroides</i>	. = +
„ 4	During life (liver and finger blood)		<i>B. icteroides</i> and another species	= ?
„ 5	Autopsy	8 hrs. after death	Variety of bacteria, including <i>B. icteroides</i>	= ?
„ 6	„	3 „	Very abundant <i>B. coli</i> ; no <i>B. icteroides</i>	. = 0
„ 7	„	0 „	<i>Streptococci</i> ; no <i>B. icteroides</i>	. = 0
„ 8	„	2 „	<i>Staphylococcus</i> and <i>B. icteroides</i>	. = +
(During life, pure <i>B. icteroides</i> from finger blood, liver, and bile)				
„ 9	„	8 „	Mostly sterile, small undetermined bacillus, and one tube of pure <i>B. icteroides</i>	= ?
„ 10	„	6 „	Variety of bacteria; <i>B. icteroides</i> not found	= 0
„ 11	„	0 „	<i>Staphylococci</i> and a bacillus which was identified as <i>B. icteroides</i> ; the account is hardly satisfactory	. = ?
„ 12	„	6 „	Mostly sterile; a chicken cholera-like bacillus, and also a not very clear account of a bacillus identical with <i>B. icteroides</i> , which soon died out	. = ?
„ 13	„	0 „	<i>B. coli</i> and <i>Staphylococci</i> ; no <i>B. icteroides</i>	= 0

In total—two positive, five doubtful, and five negative cases, a result which is, perhaps, hardly sufficient for establishing the etiology of the disease. Moreover, the study of the characters of the bacillus does not impress the reader that the same bacillus was always met with; at any rate, a considerable amount of stress is laid upon the appearance of certain colonies upon agar, which alone, without other tests, is hardly sufficient as a criterion.

1. Sanarelli, *Annales de l'Institut Pasteur*, XI, 1887, pp. 433 and 673.

Following SANARELLI came a number of publications dealing with the bacillus; of these, that of WASDIN and GEDDINGS¹ gives positive finding of *B. icteroides* in thirteen out of fourteen cases examined. Whilst they undoubtedly met with the bacillus in question, their account, especially of the fermentation reactions, is not entirely clear; it is not possible to summarize their results, for it is not always certain whether the cultures mentioned were pure or not; in several instances it is stated that contaminations such as *B. coli* were present.

The much discussed case of P. Smith² perhaps gives a clue to the asserted frequency of occurrence of *B. icteroides*, 'the present attack of illness came on the 5th of February, 1899 (W. and G.).' On the 10th, *i.e.*, 'on the sixth day of the disease,' blood was taken from the ear tip, and with this plates were prepared; these 'gave us numerous colonies of *B. icteroides*; also of two other organisms, one a colon. We offered this evidence as diagnostic on the 12th.' It is not stated whether fermentation tests were made to prove the diagnosis of *B. icteroides* within this space of two days, anyhow it would not have been possible to have repeated them. At the autopsy 'in twenty-four hours the spleen gave a pure culture of *B. icteroides*, and the blood, a culture but slightly contaminated. Other organs and fluids the same. There was observed no *B. typhosus*.'

AGRAMONTE³ does not regard this as a case of yellow fever at all; his account of it is at variance in several particulars; thus on February 4, P. Smith had 'felt sick for about a week,' or Major DUCKER's statement, 'on February 9, 1899. . . . It was then the eighth day of the soldier's illness.' It is agreed that *B. icteroides* was present; thus, 'We found *B. typhosus* and *B. icteroides* in almost pure cultures from the spleen; *B. typhosus* in pure culture from the kidney; *B. coli*, *B. icteroides*, and a non-motile bacillus from the blood; pure culture of the bacillus only from the blood of the heart.' Both accounts agree that there was malaria, and that there were no typhoid intestinal lesions. The evidence on which the recognition of the typhoid bacillus is based is not given, but this case is given so much attention here not because of yellow fever (AGRAMONTE gives the opinions of six consultants, all of whom considered that the case was not yellow fever), but from the point of view of the pathogenic relation of the presence of *B. icteroides*, *viz.*, was it merely an accidental contamination, or had it anything to do with the illness? It becomes of interest to call attention to the work of the following observers, and to remark that the bacilli of GWYN and CUSHING (*vide infra*) were extremely typhoid-like when examined by the writer, for which without care they might have been mistaken.

The more exact relationship of the *B. icteroides* was established by REED and CARROLL⁴ who found that it belonged to the so-called hog-cholera or 'enteritidis'

1. Wasdin and Geddings, *Report of U.S. Marine Hospital Service*, 1899.

2. No. 7, Wasdin and Geddings, *l.c.*, and Agramonte, *v. infra*.

3. Agramonte, Reprints from the *Medical News*, February 10-17, 1900.

4. Reed and Carroll, *Medical News*, September 9, 1898

group of bacilli. It is from this point of view that the *B. icteroides* obtains greater interest, for there is a certain amount of evidence that members of this group are capable of giving rise to disease in man apart from yellow fever (*vide* references to several papers concerning 'food poisoning' given in the *British Medical Journal*, vol. ii, 1899).

More especially interesting are the outbreaks of so-called psittacosis, apparently due to the importation of parrots from South America (Buenos Ayres). And the cases related by GWYN¹ of a case of typhoid-like disease, presumably caused by a bacillus associated to this group; and by CUSHING² who made a very careful study of the relationships of his bacillus.

To return to AGRAMONTE's account, by using the same mode of taking blood as that used by WASDIN and GEDDINGS, namely, from the tip of the ear, although not unnaturally contaminations were met with, the *B. icteroides* was never met with; this, perhaps, hardly harmonizes with the supposition that WASDIN and GEDDINGS had derived their 'success' from bacteria washed from the skin. Thirty-seven cases were thus investigated. Blood was then taken directly from vein and planted in twelve cases into broth, in seven cases into milk, and in thirty cases upon agar; only in four cases did growth occur, and in none did *B. icteroides* appear. So that the findings were entirely negative both with peripheral capillary and systemic venous blood.

AGRAMONTE gives also the result of twenty-three autopsies performed upon yellow fever cadavers; in seven of these *B. icteroides* was met with (*i.e.*, 30.43 per cent.), at the same time much contamination or invasion was present, for out of the twenty-three not one appears to have been sterile; since forms of *B. coli* and cocci were encountered. Thus *B. coli* occurred 'constantly' in the liver and was also found in kidney, spleen, etc. Bacillus 'X' of STERNBERG and *B. pyocyaneus*, were also met with. It would not be profitable to discuss the reason of this very high proportion of contamination. Furthermore, three cases which were not yellow fever (including the above-mentioned case of P. Smith) also one of stabbing and one of combined ague, rheumatism and dysentery) yielded *B. icteroides* on cultivation. Next the serum of yellow fever patients (thirty-eight) was tried for agglutinative reaction in dilutions of 1:10 without positive result.

The serum of convalescents was also tried to see whether it would protect animals against injections of *B. icteroides*. So far as the experiments go they failed to shew a protective influence; the exact method of experiment is not detailed nor is the approximate multiple of the *minimal* fatal dose of the culture given, so that the value of the trial cannot be exactly appreciated. Lastly, the effect of the serum of convalescents was tried on four cases of yellow fever; one in which the treatment only began on the fifth day of illness died, the others recovered, but the account does not

1. Gwyn, Bulletin of the Johns Hopkins Hospital, 1898, IX, p. 54

2. Cushing, *ibid*, 1900, XI, p. 156

conclusively shew that the course of the disease was essentially modified by the injections, although the scheme is by no means without hope.¹

The nett result of AGRAMONTE's work is that *B. icteroides* has nothing to do with yellow fever, and presumably appeared solely as a contamination. The relationship of the *B. icteroides* with yellow fever has been carefully questioned by the American Commission (REED, CARROLL, LAZEAR, and AGRAMONTE²); at first is recorded the examination of blood of eighteen patients withdrawn on different days of the fever (first to ninth) by means of a syringe from a vein at the elbow; the blood was put, in quantities of $\frac{1}{2}$ c.c. into 10 c.c. broth. As a rule these blood cultures were sterile of all growth, in none did *B. icteroides* appear.

Further, eleven autopsies were made on yellow fever cadavers; in all of these SANARELLI's bacillus failed to appear in the cultures. No information concerning other organisms occurring in the cultures is given.

Next, in four cases blood was transferred from yellow fever patients to non-immunes; at the same time cultures were made in broth, three of these remained sterile, the fourth yielding staphylococci. Since in each of these cases yellow fever resulted as a consequence of the inoculation of the same blood into man, the authors argue that the exclusion of SANARELLI's bacillus as the cause of the fever is conclusively determined; the only apparent sources of fallacy in this conclusion would be (1) that the bacillus was so scanty that it happened to be solely in the samples injected; this, I think, may be fairly discounted; (2) that the broth used was insufficiently favourable for the growth of the bacillus; and (3) that the bactericidal power of the blood before transference to broth or of the resulting mixture of blood and broth was sufficiently high to kill, or to inhibit the growth of, the bacillus; the recognition of this last factor has been used in the cultivation of typhoid bacilli from living patients; but it probably has no effect upon cultivations made from organs to autopsies.

In summary in the three communications³ these authors consider that the bacillus of SANARELLI can be definitely excluded from the etiology of yellow fever.

Our own observations at Parà are in accordance with this conclusion, as will be seen. The inoculation upon culture media of blood taken directly from veins (second, sixth, and seventh day) of different patients during life was only carried out on three occasions; in each case the broth used remained sterile (aerobic and anaerobic); cultures from blood from the ear-lobe were only made on two occasions; one of these yielded diplococci and tetrads aerobically, the other remained sterile. It did not seem to be a very profitable mode of research in the light of AGRAMONTE's experience.⁴

1. Finlay (Elstein and Schwalbe, *Handbuch d. practl. med.*) commenced this as a mode of prophylactic and therapeutic treatment in 1893.

2. Reed, Carroll, Lazear, and Agramonte, preliminary note, *Philadelphia Medical Journal*, October 27, 1900.

3. Preliminary note, *Philadelphia Medical News*, October 27, 1900, additional note, *Journal American Medical Association*, February 16, 1901, and experimental yellow fever, *American Medical*, July 6, 1901.

4. A number of flasks containing large quantities of broth were got ready, but owing to other observations they were left for a short time, and consequently became full of moulds and unusable.

At autopsy the technique adopted was to burn the surface of the organ thoroughly with the thermocautery over an area about 2-3 inches in diameter, the central part being more thoroughly charred; a large loop of 2.5 mm. platinum wire (13×3 mm.) was employed to convey material; three of these loopfuls was put into each tube. For fluids, such as pericardial and bile, drawn-out glass tubes were thrust through the wall after free cauterization; cerebrospinal fluid was taken in syringe by lumbar puncture after cleansing the skin with lysol solution. The liver, heart, etc., were always left *in situ* until the cultures had been laid; the spleen and kidneys were removed first (though in general I prefer to inoculate from the former before removal), Mesenteric glands, etc., were cut out with the thermocautery and held in sterilized forceps.

The primary inoculations were made into broth and dextrose broth and kept at 37° and at room temperature, *i.e.*, about 25° C.; later in some cases glycerine (5%) broth was used. Both aerobic and anerobic (hydrogen and alkaline pyrogallol) conditions were used in each case. Whenever growth occurred and was found to be suggestive of typhoid-like or colon-like bacteria, the organisms were isolated and tried on fermentation tests with dextrose, lactose, and sucrose added to litmus-sugar-free broth.

An inspection of the tabulated statement on page 501, shews that in general our media remained sterile; altogether, cultivations were tried from thirteen corpses. If we may except the instances of the lymphatic glands, where the technique is more difficult to ensure freedom from chance contamination, it will be seen that coliform bacilli were only met with twice, when those from the different organs gave the same fermentative reactions and were presumably identical (all three sugars attacked in both cases). Another case yielded signs of general coccal infection (No. 6).

Only in one case (No. 2) was a member of the 'enteritidis or hog-cholera group' met with, and then only in the bile, but in pure culture. It was very actively motile and typhoid like; it did not grow luxuriantly like many other members of the group, and herein also was typhoid-like; it fermented dextrose, but not either of the other two sugars; in litmus milk whey it grew very poorly and remained faintly acid and almost clear, alkali formation only occurred after more than three weeks; parallel cultures of *B. icteroides* in whey began to go alkaline after about a week, whilst a culture labelled 'le sage' (kindly given by Surgeon-General WYMAN in Washington) went alkaline at the fourth day after the habit of the rapidly-growing GÄRTNER type of *B. enteritidis*. It appeared that this bacillus from *post-mortem* No. 2 was rather of the type of GWYN's and of CUSHING's¹ bacilli. The serum of two convalescents had no agglutinating effect upon this bacillus 'bile' at *post-mortem* No. 2 at 1 in 20 dilution. It would seem to be legitimate to argue that if SANARELLI's bacillus were of real importance in the etiology of yellow fever, we should have met with it in more than one single isolated instance, in which it will be noted that the liver, spleen,

1. Gwyn and Cushing, *Journal of Experimental Medicine*, 1901.

kidney, heart blood, and cerebrospinal fluid all remained negative. One other point, which may be remarked upon is the comparative freedom of bacterial growth in our series of autopsies, when compared to the records of SANARELLI, AGRAMONTE, and others. This may be partly due to the shortness of the period which was usually allowed to elapse between death and autopsy, to the care in thoroughly cauterising, and perhaps to the use of a spirit bunsen burner instead of the ordinary spirit lamp for heating the platinum wire and glass tubes. The broth we used showed itself very favourable for the growth of various bacteria when growth did occur or when plantings were made from other cultures. One mode of origin of the so-called agonal and *ante-mortem* invasion of bacteria which suggested itself, was the introduction of material by hypodermic medication which is often resorted to during the later and the agonal phases of the fever.

B. MOSQUITO TRANSFERENCE

It is incontestible that Dr. CHARLES FINLAY, of Habana, was the first to undertake direct experiments to substantiate his ideas of the part played by the mosquito in the transmission of yellow fever. His method was to feed mosquitoes upon yellow fever patients (not later than the sixth day), and then after an interval of from forty-eight hours to four or five days to allow them to feed upon susceptible persons; the view was to produce a slight attack of the fever in order to produce immunity. At a delightful chat we had with the courteous doctor, on July 25, 1900, he told us many details concerning his experiments, which were commenced so long ago as 1881. Altogether, one hundred and two persons had been tried in this manner, and in seventeen instances some pathogenic effect had followed the bite; this consisted in slight fever appearing about the fifth, sometimes as late as the fourteenth day. In no instance was there a definite attack of yellow fever as the result, but Dr. FINLAY thought that a certain immunity had been conferred since only four of these persons died of yellow fever, though the cases were followed out to ascertain their after history, in some cases for four years. Naturally it was not possible to exclude intercurrent infections by thus working in an endemic centre, still the mode and kind of experiment which has since led to more definite results was laid down. The kind of mosquito used by Dr. FINLAY was the *Stegomyia fasciatus* (it was referred to in his papers as *Culex* mosquito), he selected this kind on account of its town dwelling habits.*

* It may be noted that Dr. Reed's commission not only performed their experiments with his species, but were also indebted to Dr. Finlay for the eggs of the same (*Philadelphia Medical News*, October 27, 1900). The few cases tried with such short gnat incubation, as Finlay used, all turned out negative (*i.e.*, six in number) where the gnat incubation was less than ten days, in four of which, however, the gnat had been fed on the fifth day of illness of the yellow fever patient; *i.e.*, near Finlay's six day limit. A seventh case surpassed this, as the gnat had been fed on the seventh day. These numbers may be compared with those of the negative experiments with longer incubations (*v. infra*). There is, however, a source whence may have come the relatively few febrile reactions which Dr. Finlay obtained in his cases for he states (*Handbuch d. prakt. med.*, Ebstein and Schwalbs art., Gelbes Fieber) 'one obtains a female mosquito from a house which is free from yellow fever infection.' In an endemic centre like Habana, it does not follow that an insect taken in a house of this description is necessarily uninfected; so that it is possible that there were some accidental infections amongst such cases, though it may be more probable that independent accidental infection in the city should have taken place since there was no isolation; still such difficulties were well insurmountable for a private individual, and credit should be afforded for a very astute presage of future experiment.

With great boldness, and fortunately without fatal accidents, Major REED and his comrades carried out a number of experimental inoculations of yellow fever in human subjects. These are reported in three communications, which, for reference, may be styled I, II, and III respectively.¹ The first two apparently successful inoculations were most unhappily somewhat vitiated by the accidental infection of Dr. LAZEAR, which, sad to repeat, was followed by fatal result, whilst engaged in more or less similar work to case 10 (I); this case, therefore, did not seem necessarily to be in consequence of the effect of the artificial inoculation.

In the succeeding cases efforts were made to keep the subjects away from the possibility of accidental infection. This may be summed up under three headings—(1) the presence of controls, (2) quarantining the subjects, and (3) locating the subjects outside the endemic area. The validity of the cases then depends upon the absence of spontaneous or sporadic cases amongst the controls, upon the efficiency of the supervision used upon those in quarantine, and the certainty that accidental infection might not occur from unguarded sources. It is perhaps to be regretted that more detailed account of the attempts to exclude accidental contamination were not given, especially the supervision at night. Again, the question of the isolation of the experimental camp leaves something to be desired, for instance, it is stated (II) that the camp 'Lazear' was 'about one mile from the town of Quemados, Cuba.' Quemados, it may be noted, is a straggling town, one street of which extends almost to the 'Quemados entrance' to 'Columbia camp,' in the grounds of which the experimental station, camp 'Lazear,' was situate; the railway line leading on to Marianao intervening between the two. Since the houses in this street were severely infected just previously to the commencement of the experiments, and that one next but one to the railway had been destroyed in consequence, for the judgment of the completeness of the isolation the distance from this region would have been useful. Again, the prevalence of yellow fever in the surroundings, and the possibility of introduction therefrom should be considered, as some distance further along the line a fatal case is reported from Marianao (v. Major GORGAS, November, 1900, Rep.). During the latter months of 1900 the fever seems to have been more prevalent in Habana than in the two previous years, *e.g.*, October, 1897, forty-two deaths; 1898, twenty-six deaths; 1899, twenty-five deaths; in 1900, seventy-four deaths. In November, 1900, fifty-eight deaths occurred, including cases from surrounding townships.

1. I, *Philadelphia Medical Journal*, October 27, 1900; II, *Journal of the American Medical Association*, February 16, 1901; and III, *American Medicine*, July 6, 1901.

The following gives a compact view of the successful cases :—The first column of numbers gives the reference numbers in Table I, p. 20, III ; the second, the identifications of cross reference to the cases as numbered in the separate communications :—

Number	Reference case	Incubation period. days	Day of fever on which gnat was fed	Number of gnats	Incubation in gnat. days	Total gnats	Total bites	Presumably infecting bites
1	10 (I)	3 dys 7 hrs	2, 1, 2, 4	1	12, 6, 4, 2	1	1	1
2	11 (I)	6 dys 2½ hrs	2, 1, 2, 2, 2	1	16, 10, 8, 1			
			2, 2, 2	1	12, 4, 10			
			2, 1, 1, 2, 2	1	12, 2, 4, 6, 10			
			1, 1, 1, 2	1	2, 4, 8, 6	4	4	3
3	1 (II)	3 dys 9½ hrs	2	1	15			
			2	1	15			
			2	1	19			
			3	1	21			
			3	1	21	5	5	5
4	3 (II)	5 dys 17 hrs	3	1	17			
			3	1	18			
			2	1	22			
			3	1	24	4*	4	4
5	4 (II)	3 dys 11½ hrs	2	1	19	1	1	1
6	5 (II)	3 dys 19½ hrs	3	1	20			
			3	1	21			
			2	1	25			
			3	1	27	4*	4	4
7	7 (II)	3 dys 23 hrs	2	1	24			
			1	3	12			
			1	4	8			
			1	7	5	?	?	
8	6 (II)	3 dys 22½ hrs	1	4	17	4	4	4
11	5 (III)	3 dys 23½ hrs	3	3	39	3	3	3
14	6 (III)	3 dys 2½ hrs	3	2	51	2	2	2
15	7 (III)	3 dys 6 hrs	3	2	57	2	2	2
16	8 (III)	2 dys 22 hrs	2	3	16	3	3	3

Whilst in the main the incubatory phase was so constant that the illness began during the course of the fourth day, there are two examples of considerable excess, viz., Nos. 2 and 4; in both of which the number of presumably infecting gnats was three and four respectively; thereby contrasting with cases 1 and 5, in which but a single gnat conferred the infection. Without laying too much stress upon variations of this character especially, because the experiments are naturally few in number, it is perhaps not unfair to remark that their validity depends upon the constancy of the watch kept upon the individuals after inoculation, and in the case of No. 16 during the latter part of the quarantine period.

Another point of interest is the comparative mildness of the illness produced and the absence of fatal result.* It may be noted that the naturally acquired and fatal attack, which unfortunately carried off poor LAZEAR, was ascribed to the bite of a single mosquito. In personal conversation with Major REED I gather that he attributes these results to the early stage at which the illness was recognized and treated by rest. Another point in regard to the naturally acquired fever is that in many, if not in most cases, exposure to infection leads up to, if not after, the time the patient is taken ill.

Major REED and his colleagues further showed that the blood of yellow fever patients, at any rate at the early stage, is capable of conferring the illness by the direct transference of blood from sick to healthy; thus 2 c.c. taken early on the second day, $1\frac{1}{2}$ c.c. taken twelve hours after commencement of fever, $\frac{1}{2}$ c.c. taken on the second day, and 1 c.c. taken twenty-seven and a half hours after commencement of fever all caused attacks of the fever.

In these papers the authors compare the nature of the fever to that of malaria partly because of the incubatory period of ten days or more necessary in the gnat; this, indeed, would be equally essential for a gnat inoculation of bacterial parasites, provided that the inoculation were not simply due to contaminated biting parts, which can certainly be discounted from the experiments of FINLAY in so far as he was never able to reproduce typical yellow fever in his cases. It is only natural to suppose that a bacterium must also be allowed the time element in order to multiply sufficiently to be able to give an infecting dose whether the organism passes directly or indirectly into the ejecting apparatus of the gnat. Again the direct transference of blood from patient to experimental individual was tried because (II) 'It seemed to us that yellow fever, like the several types of malarial fever, might be induced by the injection of blood taken from the general circulation of a patient suffering with this disease.' This, though undoubtedly true of the animal parasite of malaria, is also possible in the case of bacterial parasites, as is a common laboratory experience when direct inoculations are made from one animal to another.

In experiments with the transference of bacterial parasites by means of biting

* More recently, according to the daily press, some fatal results have occurred: details as yet are wanting.

insects the time factor does not seem to have been considered. Thus NUTTALL¹ shews that bugs and fleas which had been fed on animals full of plague and other bacteria were incapable of giving the disease to other animals when directly transferred to them ; whether they could have done so after an incubation period, and whether the bacteria could have been discovered in their salivary or ejecting apparatus is not known.

Another point which also reflects upon the mildness of the fever conferred is the small quantity of albumin in many in the urine in the experimental cases ; thus out of the twelve gnat infections in five the quantity is described as a 'trace,' and in one other there was no albumin until thirty-six hours after the fever had subsided.

The control cases were formed by the seven susceptible persons accommodated at the experimental station camp 'Lazear' (II), where the incidence of the fever 'was strictly limited to those individuals who had been bitten by contaminated mosquitoes.' It may then be taken as proved that *Stegomyia fasciata* (*Culex*) may be capable of conferring the disease some ten, twelve, or more days after having fed upon yellow fever patients during the first, second, or third day of the fever. The cases which were bitten by gnats which had been fed upon yellow fever cases later in the disease, viz. :—

Case 6 (I) one mosquito fed ten days before on fifth day of fever,

Case 8 (I) one mosquito fed thirteen days before on fifth day of fever,

Case (III, p. 18) one mosquito fed forty days before on fourth day of fever, proved negative. The question of how long the infective agent remains in the circulating blood, whence it can be extracted and transferred by gnats is important for combatting the prevalence of the disease by means of isolation. It can hardly be doubted that the infective agent does not remain for prolonged periods in the circulation of those who have passed through the disease, otherwise it is difficult to understand the comparative readiness with which the disease disappears in localities, or has done so in the past, without any overt act directed against the gnats. Conversely there is a tenacity with which the fever remains endemic in the Latin-American countries of Central and South America ; here there are four possibilities : one already dealt with, the continuance of the parasite in the circulation of the 'immune' ; second, the occurrence of second or further attacks amongst the 'immune,' with which may be included more or less primary attacks in the children of the 'immune' ; thirdly, the prolonged survival of infected gnats ; fourthly, that the infecting agent is able to remain alive independently of man and gnat.

Apparently it is with the third method that Dr. REED considers that the foci of the fever are maintained by proving that two gnats (*S. fasciata*) which had been kept for fifty-seven days after their infecting feed upon a yellow fever patient on the second day of illness, were capable of conveying the infection to a susceptible person

1. Nuttall, *Zur Aufklärung der Rolle, welche Insekten bei der Verbreitung der Pest spielen u. s. w.* Centralblatt f. Bacteriologie, I Abth XXII, 1897, p. 87, and *Zur Aufklärung der Rolle welche stechende Insekten bei der Verbreitung von Infektionskrankheiten spielen*, ibid XXIII, p. 625.

(Case 7, III). If this should prove to be the sole mode of persistence of the contagium, the suggested analogy with malaria ceases so far as we yet know the natural history of the latter complaint, it is rather the persistence of the parasitic agent in the human host, which is chiefly responsible for the endemicity of the disease, and in Texas fever also this seems to be the case.¹ Although in yellow fever the contagium may not persist in the circulation, its temporary occurrence in the blood of natives and their children may be an important factor. From this point of view the occurrence of second attacks or of attacks amongst the natives is of considerable importance, or, to put it more precisely, the occurrence of attacks of fever amongst these people, which, though due to the yellow fever parasite, does not manifest diagnostic symptoms of the fever, which is only to be expected in the presence of a comparative degree of specific immunity.

The following is an instance of an early second attack or remote relapse with fatal ending :—A.P.D.S., thirty-three years, taken ill thirty days after arrival at Parà ; when seen on the eight day he was much jaundiced and had a high degree of albuminuria ; there was vomiting but no fever ; the icterus and albuminuria gradually diminished, and ten days later he was discharged from the hospital. Six weeks after this he was readmitted with two days illness ; he had fever and slow pulse (T. 39°, P. 70), much albuminuria ; uraemia had already set in, and with constant hiccough he died on the fourth day of the new attack ; unfortunately no *post-mortem* examination could be obtained. In both instances the diagnosis of yellow fever seemed indicated. Two or three persons whom I have seen have told me that about a month or so after they had yellow fever they had a sort of relapse of attack, and it is possible that the monthly recurrence of 'malaria,' which is talked of in Parà, is of an allied nature. For quarantine purposes, I am disposed to think that a period of six or seven weeks should have elapsed since an attack of yellow fever before an individual should be classed as an 'immune.'

Again, it reads rather more satisfactorily that a man (Case 3, II) was nine days in quarantine, than that another was (Case 7, III) seventy-eight days, which appears rather a long time for certain knowledge of all his movements short of absolute incarceration. Still, apart from these criticisms, the striking feature of the experiments is the statement that amongst the local community *only those persons who had been subjected to artificial inoculation contracted the disease.*

The first nine experiments (I) were uniformly negative : of these an eight-day period in the gnat was the maximum, except in two cases, where it was ten and thirteen respectively, but the sources were very mild cases on the fifth day of disease. In the latter cases it is shewn that not every gnat which has been fed upon a yellow fever case during the first three days of illness is capable of conferring the fever.

1. Theo. Smith, *The Aetiology of Texas Cattle Fever*. *New York Medical Journal*, July 8, 1899.

NEGATIVE CASES

Number	Species of gnat	Date of fever of source	Days of incubation in gnat	Total gnat bites
1 (II)	<i>S. fasciata</i>	5th day, severe	11 & 14	2
	<i>S. fasciata</i>	3rd day, severe	6 & 9	
2 (II)	<i>S. fasciata</i>	3rd day, moderate	12*	4
		2nd day, well marked	10†	
		3rd day	15*	
		2nd day	13†	
4 (II)	<i>S. fasciata</i>	2nd day, severe	10*	4
		2nd day	13*	
		2nd day	17*	
		3rd day, fatal	12	
5 (II)	<i>S. fasciata</i>	3rd day, well marked	12*	4
		3rd day	15*	
		3rd day, well marked	18	
		3rd day, well marked	18	
ix (III, p. 18)	<i>S. fasciata</i>	1 day	22	12
xx (III)	<i>C. fungens</i>	?	19	5

Leaving the last two out of account, and taking the twelve successful inoculations, this gives a proportion of failure in 25 per cent. From this we may take it that there is not always a sufficient number of the parasitic agents in the blood of a patient on the second or third days to infect a gnat, so that it becomes able to pass it on when feeding on a susceptible person. This suggests the conclusion that the parasite would not be discoverable in an ordinary pair of coverglass films, supposing that one, or quite a few, are sufficient to cause infection of the gnat, for the amount of blood, etc., taken by the gnat is greater than that used for such films. Hence, perhaps, the examination of plain blood films has been hitherto without avail.

The commencement of an attack of yellow fever and the time of infection has often been said to be during the night. In view of this the report of Major REED (III, p. 21) says 'If the hour of inoculation in all of our cases should have taken place at about sunset, then with the same period of incubation, seven, or 43 per cent., would

have experienced their attacks at night.' By reducing the times of inoculation of these sixteen cases to noon and midnight and 6 o'clock morning and evening, and also considering the hours about 6 o'clock as neutral we find that if the inoculation had been at :—

6 a.m.	the onset would have been during the night	in ten cases.
Noon	„ „ „	„ three cases.
6 p.m.	„ „ „	„ five cases.
Midnight	„ „ „	„ twelve cases.

So far as this mode of argument is feasible it shews that the greatest number of onsets during the night would have been from night biting mosquitoes, infecting at midnight ; in Parà this is incompatible with *S. fasciata*, but compatible with night mosquitoes as *C. fatigans*. The next largest figure is that for 6 a.m. infections, this is compatible with the bites of *S. fasciata*.

Whilst it would not be profitable to enter into all the occupations in their liability to yellow fever danger, we were much struck by the number of bakers that we saw as yellow fever patients, thus out of five hundred and twenty-nine cases as many as thirty were in bakers ; the nature of their trade was suggestive of much exposure to mosquito bites.

As an indication of the position of the belief that the fever is usually caught at night, the following quotation from DE AZAVEDO SODRE and COUTO may be made :— ' As soon as an epidemic of yellow fever commences, many foreigners and unacclimatized persons as well as all well-to-do families, withdraw from Rio de Janeiro and Santos to Petropolis and Sao Paulo respectively, thence they travel daily by early trains to the city, and return again in the evening. Now, although they remain in the pest-laden city from 10 a.m. to 4 p.m., and eat and drink there without any precautions, they escape infection in all epidemics. Those, however, who for one reason or another have to remain overnight or for several nights in the city are often struck by the fever. This circumstance shews the necessity of avoiding spending the night in places which are infected with yellow fever.'¹

The incubation period in gnat-infected cases (III) gave an average of nearly four days with the twelve cases (three days twenty-two hours), but the last case is of interest in having so short a period as two days twenty-two hours (provided that there was no previous accidental contamination). Compared with malaria this is extremely short, for in experimental cases a much longer incubation period has been established : Tertian, sixteen to twenty-five days ; Aestivoautumnal, twelve to sixteen days.²

In tsetse disease in rabbits, the first rise in temperature is generally about the eighth or ninth day ; certainly small animals (rats) will die as early as the sixth day

1. Nothnagel, vol. IV, p. 302.

2. Fearnside, *Sci. mem. of med. officers of Indian army*, XII, 1901.

after infection, but then the number of parasites introduced is relatively far larger than would be the case probably in gnat-borne yellow fever.

In Texas fever¹ the incubation between injection of blood of sick animals and onset of fever 'may be in from six to ten days, depending upon the number of microparasites originally introduced, the predisposition and age of the animals, and the season of the year.' With inoculations by means of young ticks 'the high temperature appeared generally in fifteen days after the first young ticks had been put on the animal.

Another point in comparing yellow fever with a zooparasitical disease like malaria is the question of the fertilization of the gnats; it seems to be that the proper sexual conception of the female *Anopheles* is essential for the development of the malarial parasite; the report of the U.S. Commission is silent upon whether the successfully-infected mosquitoes were fertilized or brought forth eggs. Whilst there may be no direct connexion of the fertility of the transferring insect, with its capability of fostering an animal parasite, possibly the mere increased nutritional circumstances after a feed of blood would be sufficient for aiding the development of bacterial parasites.

Another point in which yellow fever differs from the known infections caused by protozoal parasites is the absence of splenic enlargement, which is found in malaria, Texas fever, and tsetse fly disease. Though certainly, it must be admitted, that our knowledge of diseases of this nature is too scanty for discussion.

The points then which seem at variance with a zooparasite in yellow fever are (1) the shortness of the natural incubation period, (2) the readiness and rapidity with which the parasite disappears from the blood, (3) the considerable degree of immunity² which is rapidly attained, (4) the short course of the illness and the usual absence of remote relapses or recurrences, and (5) the absence of splenic enlargement. At the same time none of these are incompatible with diseases of bacterial origin.

In regard to the uncertainty of diagnosis in some cases and their importance in the continuance of the disease, the following remarks may be made:—In Cuba a special form of fever, which is termed 'Borras' (or Fiebre de Borras), is recognized amongst the inhabitants (generally juvenile); this has been considered distinct from yellow fever by Cuban physicians. In Pará the fevers of children seem usually diagnosed as 'Febre palustre'; throughout Brazil another fever called 'Febre remittente bilioso dos paizos quentes' (remittent bilious fever of hot countries).

1. Smith and Kilborne, *Investigations into Texas or Southern Cattle Fever*, Washington, 1893, pp. 15 and 106.

2. Theobald Smith and Kilborne, p. 134; 'These experiments demonstrate the important fact that one attack of Texas fever does not necessarily protect the animal from a second attack. Of the eighteen cases seven may be said to have remained practically unaffected during the second exposure. Of the remaining eleven three died during the second exposure. . . . Hence we must be careful in giving even in these cases too much credit to the first attack in warding off the second following one. . . . But it may be laid down that as a general proposition that a single attack is not sufficient to produce complete immunity.'

'Borras' fever¹ 'is a pyrexia most frequently attacking children, though it may occur at other ages. It is characterized by vomiting—abundant or scanty—lasting a few hours; or the days of the febrile attack rarely pass without this disturbance; occasionally there is albuminuria but never gastric haemorrhage, epistaxis, melaena, petechiae, hiccough, or icterus. It may occur more than once in a lifetime; both whites and coloured are attacked—most frequently whites up to the age of twelve to fourteen years; the mortality may be as high as 30 per cent.' On the other hand, JUAN B. FUENTES² says that haemorrhages occur and that when 'icterus and albuminuria are present it may be confounded with yellow fever or icterus gravis.' So that there is some degree of uncertainty as to what borras fever is. Anyhow, it is regarded with great suspicion as being merely one of the many clinical types of yellow fever; thus in the report of vital statistics of Habana, etc., for July, 1901, by Major GORGAS, we find:—'We make the best record ever made before for July, having had only four cases and one death. Two of these cases (both of which were reported as 'Borras' and one of which died) occurred in children of Cuban parentage, born in Habana, having lived in this city continuously since birth. The physicians of Habana, as a body, do not recognize this disease as being yellow fever, and indeed, both in its symptomatology and pathology, it differs widely from the disease in the adult; but the board, to which all cases of yellow fever are referred, after careful consideration, and in one of the cases after a careful autopsy and histological examination of the organs, concluded that the two cases were yellow fever.'

In Pará it was seemingly impossible to get people to appreciate the importance of the complete diagnosis of malarial fevers by the microscope, and the impossibility of doing so in patients filled up with quinine; consequently we were rarely able to decide in mild cases of transient fever which passed as 'febre palustre.'

In one cottage I came across a family in which two children had recently died, and a third was moribund; the nearest known *Anopheles* breeding place was fully half-a-mile away and separated by trees; the parents were unaffected. Unfortunately it was not possible to make any proper examination, and an autopsy was out of the question; still it seemed clear that some intense infection was present. There was no rash or icterus there, the condition of the child seemed almost meningitic. Vomiting seemed to have been the chief symptom, besides 'fever.'

With regard to the 'bilious fever' none of the few cases which I saw diagnosed as such, agreed with the symptoms laid down by TORRES HOMEM³; they were all in recently arrived individuals, and there did not appear any definite reason why they should not be classed as yellow fever. In TORRES HOMEM's account we find that this bilious fever or 'febre amarella dos acclimitados' is very common in Rio, especially during the summer (*i.e.*, the yellow fever period), and that it is the dwellers

1. Luis Perna y Salomo, *Revista medicina tropical Habana*, Tom II, p. 49.

2. Juan B. Fuentes, *ibid*, Tom I, p. 75.

3. Torres Homen, *Estudo clinico sobre as febres do Rio de Janeiro*, 1877.

in the city that are chiefly attacked; whilst in the great foci of paludism outside the city, and especially outside the municipal limit, *febre remittente biliosa* is very rare. In giving points for differential diagnosis from yellow fever, he says that 'the presence of an epidemic of yellow fever should always form a point for differential diagnosis'; supposing that the relationship to yellow fever is more essential, the disregard of such cases would be just apt to foster the yellow fever and to ensure its reappearance.

GUIERAS¹ makes the following significant statement: 'The bilious remittent fever, that in our old text-books of medicine occupied so conspicuous a place in tables of differential diagnosis with yellow fever, has practically disappeared from the Southern Sea border since yellow fever ceased to be endemic there.' It is clear that in endeavouring to rid a neighbourhood of the yellow pest, notice and suspicion must be cast upon cases in which this other diagnosis is made, and they must be dealt with accordingly. DOMINGOS FREIRE isolated a colon-like bacillus as the cause of the bilious fever, but said nothing concerning the malarial parasite; which, however, may occur in mixed yellow fever and malaria. On the other hand, AZEVEDO and COUTO look upon this bilious fever as a clinical form of malarial ('haemoglobinuric palustral') fever with icterus. It remains to be seen whether the condition is due to some further implantation in an old malarial patient of other organisms than that of malaria; naturally the mere presence of the malarial parasites will not exclude the possibility of other factors.

It is generally stated that second attacks of yellow fever are rare, and that therefore the immunity against the disease is very complete. However, it is also commonly accepted that this immunity breaks down if the individual is long absent from infected regions, so that in reality it would appear that the acquired immunity is of comparatively short (say a few years) duration; constant exposure or rather constant reinfection is therefore essential for continuance of immunity. It is not to be expected that a typical attack will occur in a partially immune individual unless he has received a very severe dose of the infective agent.

The following example is one of a not very typical attack occurring in an individual who had had yellow fever twelve years before at Pernambuco, and who had been engaged since in and about Brazil. Taken ill suddenly in the afternoon with rigor, temperature 101°, pulse 80, and took large dose of quinine. There was no typical facies; some headache, much bilious vomiting, which continued for three days; on the third day there was a trace of albumin in the urine, which soon cleared up. Temperature and pulse:—

2nd day—morning temp., 102·6°, p. 86; afternoon temp., 102·6°, p. 88;

3rd day—morning temp., 103·6°, p. 90; afternoon temp., 103·8°, p. 90;

4th day—morning temp., 99·2°; afternoon temp., 99·4°;

after which the temperature became normal. There was no icterus. Malaria parasites were not looked for on account of the quinine, but there did not seem any reason from the clinical side to suspect malaria.

1. Guiteras, *Report of U.S. Marine Hospital Service*, 1898, p. 299.

V. OWN OBSERVATIONS ON YELLOW FEVER ETIOLOGY

On the accompanying table will be found the results of our examinations of the *post-mortem* material.

The course of our investigation was chiefly directed at first to the search for some protozoal parasite, but it so happened that, especially in the specimens of the mesenteric glands out of the first autopsy, we were much struck with the presence of a fine minute bacillus in some quantity. At the next autopsy, especially in the observation of the fresh spleen juice, we were so much struck with certain curious elongated structures of protoplasmic nature, that we spent much time in searching for these and other similar bodies in tissues of subsequent autopsies and in blood of living patients. Eventually we proved that these structures were artificial, by producing them from our own blood. About the same time we came to the conclusion that possibly the investigation of mosquitoes captured in suspected houses might give a lead for further work. Almost the first of these (a *C. fatigans*) dissected on 6th December, 1900, showed large numbers of what instantly reminded me of the bacillus we had encountered at the first autopsy, some others gave a like result. We then commenced to stain and examine the material prepared from the other autopsies for this organism particularly. Owing to the scanty numbers which were found in given specimens, this process of examining took some time, and the thorough search through the old material was not finished when we were taken ill ; meanwhile, material obtained at other autopsies was examined, and careful search again and again revealed its presence. Since there was a suspicion that possibly a bacillus found in such small numbers might be merely an accidental incomer, attention was paid to see where it could come in. The points at which contamination might occur were in the preparation of the film from the organ, this was excluded by care in searing and using recently-heated capillary tubes to obtain the juice ; the freshness of the cadaver at the *post-mortem* and the frequent sterility of the organs by culture showed that no gross contamination usually took place.

The possibility of the presence of organisms on the coverglasses from the water in which they had been washed led us only to employ ones which had been thoroughly heated by the spirit lamp on a piece of wire gauze nearly to redness just before use. Since the organisms clearly appeared in the film and not superposed to it we did not think it likely that it could have got in during the staining of the dried flamed film ; however, besides using the stain as heretofore with five per cent. carbolic acid in its constitution, we tried washing out also in carbolic, and only using recently sterilized Petri dishes for the stain and the washing. It was difficult to see what other

Number	Initials and Age		Remarks
	BACILLUS	Varia	
I	P.V.		Albuminuria. Icterus. Anuria. Vomito negro in stomach. Fatty liver.
II	C.A.	y = +	Vomito negro. Albumen. Icterus. Haematemesis. Stomatorrhagia. V. negro in stomach. Fatty liver.
III	Md. J.		Vomito negro. Albumen. Not icteric. Hiccough. Uraemic epileptiform convulsions. V. negro in stomach. Fatty liver. Spleen large, pigmented, malarial
IV	A.F.		Albuminuria. Black vomit. Yellow after death. V. negro in stomach and intestines. Fatty liver.
V	M.V.		Devoted to examination of organs in fresh state. Icterus. Albuminuria. Suppression. V. negro in stomach. Fatty liver.
VI	M.D.		Icteric. Enterorrhagia. Mania. Suppression. No black vomit in stomach. Liver very fatty.
VII	G.M.	ent	Icterus. Albuminuria. Suppression. Fatty liver.
VIII	F.S.G.	nd = +	Icterus. Albuminuria. Suppression. V. negro in stomach. Very fatty liver.
IX	G.	l = +	Albuminuria. Icterus. V. negro in stomach. Liver fatty.
X	M.B.	orrhage = o	Very obese. Not yellow. Albuminuria. Stomatorrhagia. Suppression. Stomach no V. negro. Haemorrhage in lung. Very fatty liver. Atrophic cell changes.
XI	B.C.	nd = +	Icterus. Albuminuria. Stomach coated with V. negro. Fatty liver. Spleen large, pigmented, malarial.
		l = +	
		nd = o	
		nd = o	
		r = o	
XII	J.C.	nd = +	Icterus. Albuminuria. Black vomit. V. negro in stomach. Fatty liver.
XIII	D.S.		Icterus. Albuminuria. V. negro in stomach. Fatty liver.
XIV	M.L.	nd = +	Icterus. Albuminuria. Black vomit. Reddish-brown watery content in stomach. Black coating small intestine. Fatty liver.
		l = +	
XV	G.V.		Icterus of juices, <i>post-mortem</i> . Epistaxis. Enterorrhagia. Fatty liver. Much disintegration microscopically. Spleen large, dark, malarial.
XVI	F.E.	nd = + tent abundant +	Icterus. Albuminuria. V. negro in stomach. Very fatty liver.
XVII	V.Z.	l = + ot very abundant	Yellow, <i>post-mortem</i> . V. negro in stomach. Fatty liver. Spleen large, pigmented, malarial.
			finding of small bacillus.

SYNOPTIC TABLE OF EXAMINATIONS

Number	Initials and Age	Date of Death	Day of Disease at Death	Interval between Death and P.M.	CULTURES					MICROSCOPICAL FINDINGS OF SMALL BACILLUS					Remarks	
					Liver	Kidney	Spleen	Varia		Liver	Kidney	Spleen	Mes. glo	Varia		
I	P.V.	25	22.ix.00 9 a.m.	6th	at once	—	—	—	—		+		+	+		Albuminuria. Icterus. Anuria. Vomito negro in stomach. Fatty liver.
II	C.A.	24	8.x.00 8.15 a.m.	13th	at once	o	o	o	Bile Blood Cereb. spl. fluid Mes. gland	= pure <i>B. enteritidis</i> var. = o = o + impure		+	+		Rib marrow = +	Vomito negro. Albumen Icterus. Haematemesis. Stomatorrhagia. V. negro in stomach. Fatty liver.
III	Md. J.	39	14.x.00 8.30 a.m.	6th		coliform	coliform	coliform	Blood Cereb. spl. fluid Mes. glands	= coliform = o = o		+		+		Vomito negro. Albumen Not icteric. Hiccough. Uraemic epileptiform convulsions. V. negro in stomach. Fatty liver. Spleen large, pigmented, malarial
IV	A.F.	14	29.x.00 9 a.m.	11th	at once	o	o	o	Pericard. fluid Bile Mes. gland 1 " 2 Axillary gland	= o = o = + pure = coliform = o				+		Albuminuria. Black vomit. Yellow after death. V. negro in stomach and intestines. Fatty liver.
V	M.V.	40	7.xi.00 12 noon	6th	at once	—	—	—	—					+		Devoted to examination of organs in fresh state. Icterus. Albuminuria. Suppression. V. negro in stomach. Fatty liver.
VI	M.D.	34	10.xi.00 4 p.m.	?	at once	cocci	cocci	cocci	Bile 1 " 2	= o = large bacillus and +				+		Icteric. Enterorrhagia. Mania. Suppression. No black vomit in stomach. Liver very fatty.
VII	G.M.	52	22.xi.00 11 p.m.	10th	9 hours	—	—	o	Bile	= o	Not examined owing to accident					Icterus. Albuminuria. Suppression. Fatty liver.
VIII	F.S.G.	29	2.xi.00 8 a.m.	7th	at once	o	—	o	Bile	= o	+		+		Axillary gland = +	Icterus. Albuminuria. Suppression. V. negro in stomach. Very fatty liver.
IX	G.	19	28.xi.00 8 a.m.	7th	at once	o	coliform	coliform	Bile	= o			+		Portal gland = +	Albuminuria. Icterus. V. negro in stomach. Liver fatty.
X	M.B.	29	4.xii.00 2 p.m.	4th	at once	—	—	o	Axillary gland " 1 " 2	= cocci = cocci and +	+		+		Lung haemorrhage = o	Very obese Not yellow. Albuminuria. Stomatorrhagia. Suppression. Stomach no V. negro. Haemorrhage in lung. Very fatty liver. Atrophic cell changes
XI	B.C.	21	13.xii.00 10.30 a.m.	16th	at once	o	—	large sporing anaerobe	Axillary gland Urine Mes. gland 1 " 2	= o = o = coliform = o	+	+		+	Cervical gland = + Portal gland = + Axillary gland = o Femoral gland = o Rib marrow = o	Icterus. Albuminuria. Stomach coated with V. negro. Fatty liver. Spleen large, pigmented, malarial.
XII	J.C.	20	17.xii.00 2.30 a.m.	6th	6 hours	—	—	o	Axillary gland Fem. gland Urine foul	= o = o					Axillary gland = +	Icterus. Albuminuria. Black vomit. V. negro in stomach. Fatty liver.
XIII	D.S.	18	1.i.01 2 p.m.	4th	at once	—	sporing bacillus	o	Blood Bile Urine Mes. glands o, o, o	= o = o = o = o		+	+	+		Icterus. Albuminuria. V. negro in stomach. Fatty liver.
XIV	M.L.	23	8.i.01 11 a.m.	5th	at once	o	—	o	Bile Mes. glands Axillary	= o = o = o	+	+	+	+	Axillary gland = + Portal gland = +	Icterus. Albuminuria. Black vomit. Reddish-brown watery content in stomach. Black coating small intestine. Fatty liver.
XV	G.V.	18	14.i.01 2 p.m.	9th	at once	o	o	o	Bile Axillary gland Mes. gland 1 " 2	= o = o = o = streptococci	+	+	+			Icterus of juices, <i>post-mortem</i> . Epistaxis. Enterorrhagia. Fatty liver. Much disintegration microscopically. Spleen large, dark, malarial.
XVI	F.E.	23	19.iii.01 3.30 p.m.	13th	at once	—	—	—	—		+	+			Axillary gland = + Duodenal content abundant +	Icterus. Albuminuria. V. negro in stomach. Very fatty liver.
XVII	V.Z.	20	20.iii.01 9.30 a.m.	6th	at once	—	—	—	—		+	+			Portal gland = + Colon + not very abundant	Yellow, <i>post-mortem</i> . V. negro in stomach. Fatty liver. Spleen large, pigmented, malarial.

no dash (—) = no observation.

o = negative or sterile.

+ refers to positive finding of small bacillus.

EXPLANATION OF SIGNS: dash (—) = no observation. o = negative or sterile. + refers to positive finding of small bacillus.

precautions could be taken, yet still the fine small bacillus appeared. Later the plan was tried of putting a large number of big loopsful into saline solution with a small quantity of corrosive sublimate, and after several good shakings allowing the stuff to sediment; thereby it was hoped that possibly in the lower part of the tube one might be able to concentrate the bacilli from a larger quantity of tissue than could be examined otherwise. The method is merely an application of the common technique in examining doubtful sputa for tubercle bacilli. At the same time ordinary smears were made for control. In this way we found the small fine bacillus with greater facility than in the plain smears.

At the earlier stage when examining centrifugalized blood we had sometimes examined the bottom of the tubes for bacilli, but without much success; further examinations of early cases were to have been made by allowing slow sedimentation to occur whereby time would be allowed for the heavier particles to sink first, which is not the case when slightly diluted blood is centrifugalized in a narrow tube. The method was tried in rather long tubes with the borax-boracic acid mixture used for tubercle, but no early cases were obtainable at the hospital except a man from a ship nearly all of whose crew were affected with yellow fever, a disease which he had already had some years before; besides tertian parasites in his blood, the sediment yielded a small bacillus similar to that in the yellow fever cases; a control case of malaria examined at the same time with the same batch of tubes and medium gave negative result. It was certain that he had been exposed to yellow fever infection on the ship, and it was possible that these bacilli were the consequence, though the course of his temperature corresponded with the ague infection.

Naturally we turned to Dr. STERNBERG's book, to see whether this assiduous observer had recorded anything similar to the small bacillus which we found. The following may be quoted: 'I have had my attention especially attracted by an extremely slender and long bacillus which has been very abundant in many of the smear preparations, but which has never shewn itself in my cultures (contents of alimentary canal). It is the smallest organism so far as its breadth is concerned that I have yet encountered; it is a flexible filament, as shewn by the various shapes it assumes, and may reach a length of fifty micromillimetres or more.' This turned our attention to the examination of the faeces, and, at our final autopsies, the intestinal contents, and we found an extremely fine bacillus, sometimes in extraordinary numbers, so that in a whole field of a $\frac{1}{12}$ O.E. objective there were but few other kinds of bacilli; we did not see such lengths as STERNBERG mentions; its tenuity was such that by opening the aperture of the condenser to a point at which the larger ordinary faecal bacilli were still visible, the fine one completely disappeared; in such examinations of fresh unstained faeces, no motility could be detected. Like the bacillus found in the tissues it did not stain with great readiness; thus, F.G., seventh day,

1. Sternberg, *Report on the etiology and prevention of Yellow Fever*, Washington, 1890, p. 113.

stool with mucous pieces ; film stained for ten minutes in somewhat diluted carbol fuchsin, large numbers of 'small bacillus,' but unstained ; staining prolonged overnight ; 'small bacillus' now stained and present in large numbers. Besides faeces it was also seen in small numbers in a few specimens of black vomit ; in one case (*post-mortem* 17) there were very large numbers in the duodenal contents ; the ileum, as in other cases, showing vast numbers of coliform and other faecal bacteria. In the contents of the colon, also, the 'small bacillus' was met with in abundance. Whether from the nature of the balsam used or from the cloveoil, etc., which was put in the boxes to prevent destruction of the labels by insects, etc., the colour of all our mounted specimens faded out and diffused. Since my return I have tried to re-stain these specimens (prolonged carbol fuchsin and aniline gentian violet) but for some reason, apparently, the bacilli must have lost staining power ; I have not succeeded in finding them in specimens which showed large numbers when they were first made. Further, it appeared suggestive that STERNBERG had met with an identical organism in tissues. Referring to the examination of sections of various organs, he says¹ 'The result of this research has again been negative, so far as the general presence of any particular micro-organism in the material examined is concerned. But in one case (No. IV) I found in the kidney a minute bacillus which apparently invaded by preference the glomeruli. It was not found in the capillaries generally, but a certain number of foci were found, some small, as shown in Fig. 6, and involving only a portion of the glomerulus, others involving a whole glomerulus, and the tissues immediately surrounding it. The appearance was such as one would expect to see in a case in which solitary bacilli, carried in the first case by the blood current, had effected a lodgment and established a centre of infection in tissues already, perhaps, necrotic and through which the circulation had ceased. The latter supposition seems to be justified by the fact that there were comparatively few of these foci, whereas if they had been established while the circulation was still going on, we would expect to find numerous secondary foci and a certain number of bacilli in the neighbouring vessels. Moreover, there was no evidence of inflammatory reaction as a result of this invasion of the tissues by parasitic organisms. I am, therefore, of the opinion that this is some ordinary saprophyte which had effected a lodgment in the kidney, possibly during the last hours of life when the vital resistance of the tissues was slight, or when as a result of the blood stasis in the organ local necrosis had already occurred at certain points before death.'² (It may be noted that this autopsy was performed one and a half hours after death ; whether the interpretation given is the right one does not necessarily follow). 'It is quite probable that during the last hours of life a certain number of micro-organisms from the intestine succeed in passing through the enfeebled tissues into the interior of the capillaries and are carried away by

1. *Op cit.*, p. 138.

2. The condition described is, perhaps, in accordance with the distribution of an anaerobe.

the already slowly moving blood stream to distant organs, where they may establish centres of growth even before death occurs, or are at least in position to take possession of the field, as soon as the vital spark has been extinguished. In the case in question, I believe that the true explanation of the presence of the organisms described is that suggested, for I have not found in the other cases examined any similar collection of bacilli, and can, therefore, not attach any importance to the observation so far as the etiology of yellow fever is concerned. In Berlin I fell upon a little group of slender bacilli in a capillary of the liver, and recently have found a similar group in a preparation of skin from a yellow fever patient' The bacillus above described, present in a single case, is then the only micro-organism found in the material obtained in Havana in 1887, so far as liver and kidney were examined.' When in Pará we thought that possibly the discovery was due to accidental successful staining of the bacillus which we met with; since my return staining of sections (paraffin) of the material obtained has not been successful in revealing groups of bacilli as had been hoped; some suggestive appearances have been seen after heavy carbol fuchsin, aniline fuchsin and violet staining, but nothing definite enough for satisfactory recognition; it is possible that the xylol, etc., used for the embedding has caused the same change as has occurred in the faecal specimens.

In his conclusions² Dr. STERNBERG remarks: 'Some of the micro-organisms present in the dejecta of yellow fever patients, as shown by stained smear preparations, have not developed in the cultures made, either aerobic or anaerobic. One extremely slender filiform bacillus, which can only be seen with high powers, and which is quite abundant in some of my preparations, has never been obtained in the cultures made, and, no doubt, there are others of the same category.' Attention may also be called to some of the photographs given by Dr. STERNBERG; whether the organisms represented are the same as the other small bacilli cannot be said; anyhow the sources of the cultures were not pure, and the investigators not very expert bacteriologists. On Plate XV, Fig. 6, is a figure of a fine bacillus found in a sample of DOMINGOS FREIRE's vaccine (which was supposed by that author to be a pure culture of his 'cryptococcus'); the specimen was stained with gentian violet and does not appear to have been coloured very intensely. On Plate III, Fig. 4, there also occurs a small bacillus concerning which he writes³: 'is from a slide mounted by Dr. ANGEL GAVINO YGLESIAS in Dr. CARMONA's laboratory. Associated with the large bacillus shown in the photograph, there is another slender bacillus in smaller number, which is seen on looking over the slide' (it also appears more faintly stained). Dr. STERNBERG does not appear to have thought of connecting these various fine bacilli with one another; it is not possible for us to identify them as one and the same organism, but the quotations are of some interest in connexion with our own observations.

1. *Loc. cit.*, p. 140.

2. *Loc. cit.*, p. 222.

3. *Loc. cit.*, p. 164.

With regard to cultivating the small fine bacillus, our attempts were practically a failure. We used to cut out several of the mesenteric glands by means of the thermocautery and introduce them into the broth tubes by means of recently-heated forceps. In two cases we got a growth (in one case pure and in another case with another bacillus) in this manner; the tubes were placed in hydrogen with pyrogallol and alkali. In the former case there was no apparent change in appearance, but on breaking up the gland large numbers of apparently growing 'small' bacillus was found. In another case a mixed culture of *Staphylococci* and the small bacillus was obtained; subcultures in these cases failed to give any growth of the small organism. It is, perhaps, noteworthy that in a large proportion of these whole mesenteric gland cultures no ordinary faecal, etc., bacteria grew, though from the large size of the pieces, it might have been anticipated, that accidental contamination would have occurred, or that SANARELLI's bacillus would have appeared. It appeared desirable to search for a medium which would be favourable for the growth of the organism, and the last thing done before being taken ill was to make several brews of tissue from the last autopsy and also to prepare milk tubes. Upon returning to work these were full of moulds and could not be utilised at the last two autopsies I was able to obtain. It seemed then that a peculiar small fine bacillus was to be found in the organs of yellow fever cases, in the contents of the gut, in local spots presumably gnat bites on the skin, and in the stomachs and salivary sac (or oesophageal diverticula) of many of the gnats examined. Piecing this altogether, it suggested that the fever consisted in a gnat-borne and introduced infection, and that the small bacillus was the active agent. It may be noted that FINLAY in his original mosquito theory looked to the inoculation of a bacterial parasite (a tetracoccus) by means of his mosquito.

As a means of seeing whether a recognizable bacillus could be traced in the mosquito, a number of preparations of the stomachs and salivary (or accessory) sacs were made from mosquitoes which were caught at the leprosy asylum at Parà inside the cottages of the lepers; some of these had obviously been feeding upon the serous subcutaneous exudation rather than actual blood. These specimens were made just before coming away, and were not stained for the leprosy bacilli; now, unfortunately, they have become mislaid. It may be added that by the notes made of the appearances in the fresh state of the organs of these mosquitoes they were all devoid of the small bacilli found in other places.

Soon after we had found the small bacilli in the brown night-biting gnat, *C. fatigans*, we received the first report of Major REED's Commission, in which the day-biting *S. fasciata* was incriminated; the proof then was slight, and we thought that possibly their successful cases were due to the accidental bite of a night mosquito like ours. That the *S. fasciata* can convey the disease has since been proved. It seemed probable that if a bacterial parasite was concerned, and that subcutaneous

introduction as by gnat bite was necessary for reproducing the fever in man, it might be that the bacillus could be swallowed by the mosquito from other sources than an actual bite on a yellow fever patient. A number of a pupae taken from a cesspool (species *C. fatigans*) were tried, but the results were not satisfactory from the external contamination and the extensive cytolysis proceeding in the creature. Another attempt made, was to catch mosquitoes coming directly up a large ventilating pipe from a sewer, but the whole thing was washed away by a rain shower. It seemed possible that a feed of blood might be requisite to supply pabulum for the growth or spread of the bacillus within the body of the gnat.

The implication of a sewage-loving gnat, like *C. fatigans*, and the fertile source of it in foul water and cesspools was thought possibly to be an explanation of the outbreak of cases of yellow fever in apparently isolated and spontaneous manner. Altogether about eighty mosquitoes were dissected; but all of these were not examined for the small bacillus; the method adopted was to remove the salivary sac and also the stomach by dissection under a lens; examine in the fresh state between two coverslips in saline solution, and then by drawing the glasses apart they were ready for making stained specimens. Out of thirty-five individuals of *C. fatigans*, small bacilli, sometimes in large numbers, occurred sixteen times; out of thirty individuals of *S. fasciata* it occurred only six times. These were insects caught in suspected houses and on a ship with yellow fever. Preparations were made for rearing insects from the egg in clean waters, so that when fed upon patients there would be no possibility of chance contamination. Although several attempts were made we never succeeded in getting the species *C. fatigans* beyond the young larval stage; with *S. fasciata* there was no difficulty, the first lot began to hatch out just as we were taken ill, and afterwards a new lot was started; when they were ready I was unable to get hold of an early case to feed them upon. So that no observations upon reared cleanly mosquitoes which had actually been fed on yellow fever cases were made.

As a working theory of the pathology of yellow fever, derived from these observations it was thought that:—

Supposing the 'small bacilli' found in the 'typical bites,' organs, salivary sacs, and stomachs of the mosquitoes and in the intestinal contents were identical in nature, and that they were the acting cause of yellow fever, the following working theory of the fever could be formulated. At first a subcutaneous introduction of the bacilli by the gnats (which might have derived them from patients or by feeding on other, *e.g.* faecal, material); affection of the superficial lymphatic glands (in which case the infection would seem to take place more frequently in the upper than in the lower extremity, as judged by the intensity of the lesions in the glands of the former); generalization through the system, giving rise to the influenza like first period of the fever; localization of the bacilli in and about the intestinal area, resulting in

absorption and distribution of toxic products causing the symptoms of the second period of the fever. It may be remarked that many writers (FINLAY and others) have laid stress on the difference between the onset and the second stage, and have variously suggested that the latter stage is due to some different cause from the first. Although symptoms of a severe character might arise from the elaboration of the specific toxic products in and about the gut as a consequence of a localization from a generalized infection it would not follow that an original infection could be established by way of the gut (compare experimental feeding and injection of many bacteria).

Prolonged cases, such for example as No. XI (Synoptic Table), and others less prolonged are perhaps due to multiple infections during the period of the illness.

TREATMENT

According to the figures (*Pará Medico*, January, 1901, p. 70), five hundred and fourteen patients were treated at the isolation hospital for yellow fever or Hospital Domingos Freire, of these one hundred and ninety-three or 37.5 died. This apparently is a high mortality rate, moreover the figures cannot be judged directly for two considerations: first, a large proportion of the patients are only sent to the hospital late in the disease, and not infrequently in an almost moribund condition (a few have arrived at the hospital actually as corpses), so that the mortality rate is no satisfactory guide; and secondly, the five hundred and fourteen patients include *all* admissions to the institution, so that the death-rate of the yellow fever cases is still higher, especially from the bed cards in which cases of slight mild yellow fever are recorded as gastric or perspiration or malarial fever, as well as those indubitable cases of malaria in which we found the malarial parasite.

Theoretically, the treatment is a 'hydrotherapie;' water to drink (plain, or Vichy or Apollinaris), copious enemata of normal saline, and, if bad symptoms ensued, hypodermic or intravenous infusion. Practically, a number of drugs were given over and above an initial purge of castor oil and calomel; aconite, digitalis, and belladonna were favourites at the early stage, and lactate of strontium later, this was supposed to be 'good for albuminuria,' but it did not appear to have any effect. Cold baths or packs, too, were much used, and were regarded by us as heralds of a possible autopsy. In the uraemic condition hypodermic injections of trinitrine, strychnine, etc., were administered; it need hardly be said that they gave no highly favourable result.

Judging from results one has seen, and without any personal care of cases, it would seem that the most important thing in treatment is to keep the patients in bed, and not allow them to leave it any more than one would in a typhoid case. Next, if the patient became infected in the house in which he is laid up, he should be removed from it at once, so that he may not receive a constantly increasing supply of the infecting agent; it is only reasonable to suppose that what otherwise might be a

mild case may become extremely grave if further and repeated infection has been allowed to occur. But if removal is to be carried out it should be done at once, and if the patient has been ill for say four days, he should not be exposed to the risks of disturbance by removal. In a well-ordered town which it is desired to free from the malady, there can be no question that all cases should be isolated, and that isolation should be effected promptly if it is to be of any use as well as non-injurious to the patient.

It does not appear that drugs are of much value ; for cases treated in this wise from the first are not saved from a fatal result, and it seems wiser to withhold from them altogether, with the exception of laxatives. It seems of importance to induce, or at any rate not to interfere with sweating, as by chilling the skin, cold bathing, and the like ; I think that care should be taken to supply blankets and other means to avoid chilling, and especially application of vapour baths or warmth. Except agonal sweats, the fact that a patient is sweating greatly increases the favourableness of the prognosis. The administration of plenty of water, in the form of saline enemata, or, if necessary, directly into the system, seems good ; whether the former should be given cold, as I have seen it employed, is perhaps a matter for grave doubt. The temperature was rarely a source of anxiety as such in the cases seen ; if it can be reduced by inducing sweating without drugs so much the better.

There can be no doubt that the patient ought to be protected from mosquito bites, for by those of already infected insects he may have added quantities of the infecting agent, and also he may contaminate otherwise uninfected insects and cause danger to his neighbours.

Just as malaria has been stamped or rather died out in England without the disappearance of the *Anopheles*, or of any very direct attack against them, so yellow fever has been stamped out in places without dealing with the mosquitoes ; thus, yellow fever does not appear in the British Guiana reports, and has practically been absent in Jamaica since 1897 ; again, instances are to be found in the southern part of the United States and Porto Rico. At the same time, it must be admitted that the method adopted in Havana by the Americans for yellow fever, and at present being conducted on the West Coast of Africa by our own countrymen for malaria, is by far the most reasonable method of clearing a neighbourhood of a gnat-borne pest, especially a town disease like yellow fever.

No prophylactic inoculation has as yet been discovered for yellow fever, though many have been vaunted. It is clearly too risky to make use of artificial gnat infections. The only scheme which suggests itself as practicable is on the lines of the useful modes employed in some diseases of animals (as rinderpest), in which a dose of infective material is given with a dose of serum of an immune animal (*i.e.*, blood of early yellow fever patient mixed with serum of a convalescent).

VI. NOTES ON POINTS OBSERVED IN YELLOW FEVER

A. TYPICAL BITES

One of the cases seen in Cuba called our attention to a lesion of the skin which in our notes we referred to as 'typical bites.' These are small rounded rather purplish petechia-like patches some two or three millimetres in diameter, surrounded by a pale zone which makes them the more prominent; they are without local swelling. In Parà we have heard them called 'petechiae,' but on pressure they can be almost completely banished so that the chief part of the lesion is a vascular dilatation; also on the *post-mortem* table they are represented by palish rather violet patches which are not very prominent. In distribution they are found on exposed parts especially wrists and ankles, but not infrequently occurring as high as the elbow and up the leg. We met with an occasional case in which they were distributed more generally over the body; thus one case is noted as covered with 'typical bites'—on 'face, forearms, shins, and ankles, also chest and abdomen and a few on back, thighs free.' We rather tended to think that the prognosis was grave in cases in which they were abundant; this particular case recovered after a severe attack. It may be mentioned that the pauper in Parà is often not very thoroughly clad, and at night perhaps any part of the frame may be exposed to the attacks of mosquitoes. In general the face escapes so far as very obvious lesions are concerned, but close inspection sometimes shews quite minute punctiform dot spots (it may be noted that at one autopsy the cervical lymphatic glands were examined and found to be deeply congested and haemorrhagic) also with a circumferential pallor.

They do not occur always, probably in about a quarter of the cases (they are noted in twenty-three out of ninety-four cases). Besides this many patients are seen to have been freely bitten as evidenced by the lesions and complaints.

There are several questions of interest concerning these 'typical bites'; in the first place they were only observed in yellow fever cases, so that it may be presumed that they have something to do with the fever. If they are lesions caused by gnat bites are they due to the bite of a particular kind of gnat? As judged by the bites we received they are not caused either by *S. fasciata* or *C. fatigans*. Are they due to the bite of an uninfected gnat, in a person suffering from yellow fever in whom the vascular dilation and haemorrhagic tendency are present? The answer here is, I think, in the negative since the patients in the yellow fever hospital were abundantly bitten by the above kinds, but typical bites did not occur after admission as far as was observed; judging by the fewness of the cases of infection derived at the hospital and the late stage of the disease at which the patients were often admitted, the gnats about the place were probably mostly uninfected.

The conclusion which we thought probable was that they were the local lesion caused by the introduction and local development of the parasite of yellow fever, and that the condition found in the lymphatic glands was of the same nature. The mode of proof by watching the progress of the bite of an infected gnat upon an experimental case could not be made, and unfortunately the Commission under Major REED do not refer to the matter.¹ Another mode of proof was by determining the presence of the yellow fever parasite in the locality. After scrubbing the skin with lysol and then with spirit, the spots were stabbed with a lancet, and the blood, which freely exudes, was taken directly into a fine sterile capillary without touching the skin. Examination of specimens thus taken were examined in the fresh state, and also stained with carbol fuchsin. In the stained condition a few small, fine bacilli from 2 to 6 μ in length were found in scanty numbers, sometimes in tiny groups of four or five, sometimes singly; besides these bacillary forms sometimes small coccoid bodies in pairs or singly could be found. It was suggestive that these might be the spherical shape assumed by the bacilli in consequence of the bactericidal action of the blood, especially from the specimen of a late case (*P.-M.* 11), who had many typical bites when admitted on the fifteenth day; in these some little groups contained, apparently, stages between the complete bacilli and the coccoid forms. Here there is, of course, a risk of contamination with skin microbes. At a later date after my illness I examined some bites (which were rather swollen and due to the entrance of several *C. fatigans* during one night through neglect of closing the mosquito net); the blood obtained contained many 'polynuclear' leucocytes, in some of which ingested bacilli, similar to our small bacillus, were found, as also in the salivary sacs of the gnats themselves; this was very suggestive of an introduction of bacilli by the gnats (Plate VIII, Fig. 2).

B. LYMPHATIC GLANDS

Since it appeared likely that the infection of yellow fever was introduced by some such agency as the gnat, it was possible that, the infection being local and superficial, there might be some signs of lesion about the corresponding superficial lymphatic glands. Our attention was more particularly attracted to this point by the marked enlargement, deep congestion, and juiciness of the axillary glands in our fourth autopsy, on October 29, 1900. After this, the investigation of the axilla became a constant feature of the clinical examination of patients; in some cases the inguinal and femoral regions were also felt, but comparatively little attention was paid to these owing to the obvious source of fallacy during life.

In forty-four cases of undoubted yellow fever, palpable and marked enlargement of the axillary glands is noted thirty-nine times, questionable enlargement three times,

1. It is obvious that if a single bite has caused the disease, the lesion, even if 'typical,' might be overlooked. I must frankly own that I should have anticipated finding the small bacillus in greater numbers in the axillary glands.

and no palpable glands only twice, in one of which the inguinal glands were also negative; of the other there is no note. Of twelve doubtful cases, axillary affection was noted in eight, and none in the remainder. In a number of other cases in which malaria was present, it was suspected that yellow fever infection was also at work. At the general hospital a number of odd cases were examined, as a rule these were negative; however, one batch of seven malaria cachectics gave two with glandular enlargement, one unilateral and one bilateral.

The general plan of palpation was to raise the arm from the side and thrust the fingers as high as possible in the axilla, put the arm to the side, and gradually work downwards. In some cases the enlargement was only on one side, in others on both; in a few cases there was distinct tenderness, but on the whole this was unusual. My colleague made observations on a number of cases from day to day, but the records of these have been lost; the general result was that the glands diminished and became unpalpable as the patient recovered. (One record in the notes gives enlargement on the fifth and eighth days, which had lessened on the tenth, and disappeared on the twelfth day). In my own case I have distinct recollection of finding distinct enlargement on one side about the first or second day; after convalescence there was nothing palpable. I may note here that previously, on October 23, I noticed that my right bicipital gland was enlarged and tender, and remained so for a few days, there was not sufficient general disturbance to take the temperature; it was ascribed to the effect of a mosquito bite without marked primary lesion. Supposing that this really was an extremely mild yellow fever infection, it would account for the mildness of the attack which I had some months later, when my comrade—certainly infected at the same time, as he was taken ill less than twelve hours after me—had so severe an infection. At the same time it may be mentioned that we met with a few cases of husband and wife in which both were taken ill on the same day, and yet the one died of acute yellow fever whilst the other had so slight an attack that without the circumstantial evidence it would hardly have been possible to make a diagnosis. Naturally, in these questions individual susceptibility cannot be entirely discounted.

The following is a summary of the conditions found at autopsy :—

Post-Mortem	Axillary	Femoral	Mesenteric *
4	Marked large. Deeply congested. Very juicy.	Seem large. Rather pale. Juicy.	Swollen and pink.
5	Large and massy. Not markedly injected.	? Enlarged.	Large and pink.
6	Much enlarged. Dark, congested. Juicy.	Large. Pale. Juicy.	Not particularly enlarged. Pale.
7	Much injected. Much enlarged.	Large. Juicy. Pale.	No note.
8	Enlarged. Injected.	Large. Pale.	Rather large. Pink.
9	Much enlarged. Deeply red.	Seem large. Not reddened.	Large. Pale.
10	Large : Somewhat reddened.	—	Not apparently large.
†11	Markedly large : Rather red.	Large, but not injected.	Larger than normal. Some much reddened.
†12	Markedly large. Some deep red.	Large : Somewhat reddened.	—
13	Marked large and red.	Apparently large. Not much injected.	Very distinct, and mostly much reddened and large.
14	Much enlarged and reddened.	Perhaps large. Pale.	Rather large : Not very red.
15	Large : Rather red.	Rather large.	All much enlarged, and rather pink.
16	Much enlarged and very red.	—	Enlarged, and rather pink.
17	Much enlarged. Deeply injected.	—	Large.

With regard to the enlargement and sometimes intense inflammatory condition of the axillary glands, it may be that it is solely due to the irritation of poisons (or bacteria?) introduced by gnat bites, independent of yellow fever, or that it is due to the absorption of the specific cause of yellow fever. The principal locations of gnat bites may be said to be the hands, head and neck, and the ankles ; it is curious to note that in our autopsies the femoral group of glands were never injected with the intensity seen in the axilla and neck.

* Berangen Ferand says that enlargement of mesenteric glands in yellow fever is due to typhoid infection (*Traité de la Fièvre Jaune*, p. 61. Paris, 1891) : if this were the case we could hardly have failed to cultivate the typhoid bacillus, or to have some typhoid ulcers.

† In these two the cervical glands were looked at, they were very deeply reddened, and some had hæmorrhages.

Naturally if there is any specific connexion between the glandular enlargement, which may be felt clinically, and yellow fever the point might be of diagnostic value. In Parà it was not possible to make satisfactory control observations ; especially in mild cases of fever, in the absence of any distinguishing mark, the discovery of enlarged glands did not lead to help—many such not included in the figures given above were seen. One question upon which no information was forthcoming is what is the condition of the palpable glands in malaria ; since being in Parà I have asked a number of physicians who have had considerable experience of malarial cases, but the axilla never appears to have been examined. In respect of this the following quotation may be of interest. A. C. SMITH¹ says :—‘ My attention has been attracted a number of times within the past dozen years to a group of symptoms which I have never seen described in any text-book . . . The group consists of inguinal bubo associated with malarial fever ; the bubo being most commonly non-suppurating and the fever of the aestivo-autumnal type, though not invariably so. The bubo, in the cases which I report, occurred without suspicion of venereal infection and was clearly secondary to the fever and dependent upon it.’ It may be remarked that the author speaks of the inguinal and not femoral glands.

Information and observation then is wanted in non-malarial and non-yellow fever localities which are mosquito ridden, and also in malarial neighbourhoods which are free from yellow fever.

C. URINE

In conversation whilst in Cuba, I think Major REED mentioned to us that they had sometimes succeeded in finding tube casts in the urine, although, chemically, the presence of albumen was not apparent. We noticed that reaction for albumen could, sometimes, be obtained by the use of picric acid when other reagents failed. About ten days after my recovery, I found that, although no reaction was obtained with nitric acid, boiling, and the like, a distinct precipitate which did not clear up on heating was obtainable with picric acid. (When very small traces are tested for I fancy that it would be best to heat the urine before, rather than after, the acid has been run on, for the agitation prevents the formation of a ring.) Thinking that, perhaps, this result might be due to the presence of some proteid of a soluble nature such as a proteose, a number of specimens from patients were examined. The following are instances of the results obtained :—P. well-marked, rather acute yellow fever. Sample of urine boiled with drop or two of acetic acid, coagulum allowed to settle. When cool, filtered. Filtrate gives a good ring with superimposed absolute alcohol, on shaking this it dissolves. Addition of an equal volume of absolute alcohol (ethylic) does not give a permanent precipitate, which only commences when two

1. A. C. SMITH. *Inguinal Bubo as a Complication of Malarial Fever*. *New York Medical Journal*, LXXIII, June 22, 1901, and *American Medicine*, vol. II, no. 1, p. 38.

volumes have been added ; three volumes give increase of the precipitate. The clear filtrate from this still gave slight haze with more absolute alcohol. This precipitate was collected and found readily soluble in water ; the solution gave xanthoproteic reaction with nitric acid and ammonia strongly, and with weak copper sulphate and caustic soda a pink biuret reaction was obtained.

F, E, fatal yellow fever ; three days before death. Urine loaded with albumen ; in this case no acetic was added before boiling, otherwise similar procedure, the precipitate resulting from large addition of absolute alcohol (four volumes) was collected with the centrifuge and dissolved in a small quantity of rainwater. Clear yellowish solution, frothing on shaking, obtained. This, like the original filtrate, gives no reaction with boiling, with or without acetic or with nitric acid. With picric acid, and with acidified corrosive sublimate precipitation occurs ; also concentrated ammoniac sulphate and saturated sodium sulphate, when acidified with acetic acid, both give a good ring. It also yields a strong xanthoproteic and beautiful pink biuret reaction.

In other cases saturation with ammoniac sulphate was tried in order to precipitate the presumed albumose, after boiling with acetic acid and filtering. In order to avoid the urates the crystals of the salt were generally added, gradually, with intervening filtrations ; on testing the filtrates with nitric acid and ammonia, an orange xanthoproteic reaction could be obtained until the point of complete saturation.

Owing to the pigmented urates, and often biliary pigments, much difficulty was experienced ; still in some cases the resulting final precipitates when dissolved in water, besides giving a good colour with nitric acid and ammonia, sometimes gave a pink colour with the biuret reaction. Picric acid also gave precipitates, permanent on heating ; whilst boiling, with or without acidification, nitric acid and potassium ferrocyanide and acetic all failed to give signs. A number of attempts, such as the use of saturated ammoniac sulphate, were tried for clinical purposes, but the large amount of urate present, especially in acute cases, shewed that these means were valueless.

In conclusion, it may be said that a more soluble form of proteid appears to be present in the urine in yellow fever. This is precipitable by higher grades of alcohol after the removal of the less soluble proteids, if present, and is also thrown down by ammoniac sulphate in saturation. Unless it is some alteration product of the more precipitable proteids, it appears to belong to the proteose class ; it remains in the urine after the more precipitable proteids have ceased to be passed, and also occurs in slight cases in which there is never any or only slight traces of these bodies. The use of picric acid and heat seems the best mode of showing its presence for clinical purposes. How far it can be used for the differential diagnosis of yellow from other fevers must be left to the future.

When the urine contains large quantities of albumin, the simple addition of acetic acid or a few crystals of neutral salt as sodium sulphate will bring down much

precipitate. The amount of coagulable proteid in almost any very severe case is extraordinary ; by the simple application of heat the solidification is often so great that the test tube can almost be inverted with safety.

Ehrlich's 'diazo' reaction. ALBERTINI,¹ from an examination of one hundred and forty-two cases of yellow fever, found the reaction negative in one hundred and thirty. The test being made from the second to the tenth day of illness concludes that the reaction is not given in simple cases of yellow fever.

Dr. MYERS tested a number of cases, chiefly at the earlier stages of the fever, also with negative result ; some further cases which were examined also gave negative reactions during the first week. In two cases in which there was malarial infection and possible yellow fever as well persistently gave positive reaction up to the twenty-fourth and twenty-eighth days. Two cases of yellow fever, in which there was prolonged jaundice, gave a fine deep-purple on the addition of the sulphanilic mixture ; this gave place to a good crimson on adding ammonia. Another case, with highly icteric urine, gave a deep-claret colour, which bleached to a dirty brown with ammonia. Several other deeply jaundiced urines failed to show either of these phenomena ; it may be added that many urines were tested at the same time so as to control one another ; a dozen malarial cachectics also gave negative results. It may be concluded that the test is of no value for the diagnosis of yellow fever.

D. KIDNEY, SPLEEN, ETC.

Kidney. The histological examination of the kidneys of the specimens is rather suggestive that the anuria is caused by an actual plugging of the tubules, and the hyaline class of cylinder seems to be especially abundant in the cases where the bladder was noted as absolutely empty at autopsy. In other instances (as No. 11), where the bladder was found full, although there is much disorganization of the kidney cells and a good deal of granular contents in the tubules, there was a marked paucity of condensed material in the form of definite casts. The casts seen at the bend of the Henle's loops look particularly likely to become hitched and cause obstruction. Moreover, the tubules and capsules shew signs of dilatation, as if there had been distension of a mechanical nature ; and compared to a specimen hardened under the same conditions, in which the cause of death was due to some kind of focal liver infection and not yellow fever, and in which there was no kidney mischief, the glomeruli seem plump and nearly fill the capsules, the glomeruli in the anuric yellow fever cases are shrunken and withered and their nuclei often irregular. Whether, when anuria sets in, it would be possible to dislodge the plugs by some method of massage, so that they might be assisted to pass along the tubules, and whether too much bruising would be caused by such treatment of an already damaged organ, the

1. Albertini, *Revista de medicina tropical Habana*, vol. I, 1900, p. 86.

extreme hopelessness of the condition might make it worthy of a trial. At the same time free and copious administration of water in the form of large subcutaneous or intravenous injections and enemata would be requisite to induce a flushing of the tubules if possible, and also vapour baths or 'hot pack.'

Spleen. The spleen does not appear to have been much examined in yellow fever. At all our autopsies with the exception of the four with malarial condition, the spleen was plump and turgid, though not enlarged; on section it was dark and usually much blood exuded. Microscopically the marked feature is the large number of large active macrophage cells¹ in the pulp; some much vacuolated and mostly containing remnants of nuclear material. It is clear that some great destruction is proceeding. Another feature is the condition of the adenoid tissue around the smaller arteries. I have to thank Dr. GLYNN for the loan of a normal specimen for comparison, and his kindly afforded experience. In many places the small arteries are devoid of any adenoid sheath; in other places where it is retained the borders are more ragged and less well marked than in the normal organ; at the same time the lymphoid tissue is much invaded by macrophage cells mostly filled with vacuoles, hyaline pieces, and nuclear remains. Curiously enough here and there these cells have distinctly ingested lymphocytes, of this there can be no doubt where a distinct vacuole surrounds the ingested structure. In the normal organ a few relatively small macrophages are to be seen, but they do not shew the activity of those of the yellow fever cases.

Liver. The chief point of interest, beyond the fatty and atrophic changes which have already been described, is the frequently large number of leucocytes, especially 'polynuclear,' in the capillaries of the liver. The same is true of other organs, kidney and spleen, but perhaps not so striking.

Blood. It is stated in many books that there is an early yellowness, which is due to the presence of dissolved haemoglobin in the blood plasma. My colleague undertook the spectroscopic examination of the numerous serum and citrated plasma samples which we obtained in the course of our centrifugalizations for microscopical purposes. At no stage of the disease, early or late, was there the slightest indication of the oxyhaemoglobin bands. We concluded, therefore, that the so-called 'haemopoietic' icterus was not due to the presence of dissolved haemoglobin.

Another statement is to the effect that the blood of yellow fever corpses does not clot well. We found that the blood taken into test tubes from the heart at autopsies made *immediately* after death clotted perfectly well and firmly; we supposed that, possibly, the statement originated from the observation of blood which had been exposed to *post-mortem* change within the vessels, and to the action of various bacterial invaders.

With regard to the counts which were made for determining the abundance of leucocytes in the peripheral blood, it can only be said that there is a marked

1. Many of these contain granules giving the blue iron reaction with potassic ferrocyanide and acid.

leucopenia or scantiness of leucocytes in the end agonal stages of the disease, this seemed to be an indication for the worst prognosis. The leucopenia probably, mutually, explains itself, and the great abundance of leucocytes found, *post-mortem*, in the vessels of the internal organs.

E. CASES OF ILLNESS AT INSTITUTO LAURO SODRE, AND OUR OWN INFECTION

Case A. Little boy had occasional attacks of fever ; these were stated to occur each month, according to the moon ; prophylactic quinine was given a few days before full (? new) moon ; no precise examination was made ; the child seemed somewhat anaemic, compared to his twin brother.

Case 1. Sept. 11. Febre de transpiracao o da acclimitasao.
 „ 2. „ 15. Yellow fever.
 „ B. „ 20. Quartan malaria.
 „ 3. Oct. 1. Febre amarella gastrica.
 „ 4. „ 17. ?
 „ 5. „ 18. Febre amarella.
 „ 6. „ 28. Recurrent in bed several days.
 „ 7. About this time in next chalet to ours.

On July 16 a man died of yellow fever ; he was an occupant of one of the chalets ; we were not able to obtain many details, so that the question whether he could have formed the original infecting case could not be determined.

Case 1. Robust middle-aged woman ; arrived from France, September 1, 1900 ; came straight to Institute ; lives in room at stables, without any civilized conveniences ; has not used a mosquito net ; takes meals at chalet G. with the G.'s.

Sept. 11. Quite well at 9 p.m. ; taken with some shivering about midnight ; vomited bilious matter, and severe headache.
 „ 12. Severe, intense frontal headache ; no pain elsewhere ; facies, nil ; chest, nil ; abdomen, nil ; tongue, flabby ; skin, moist, sweating, slightly ; temp., 38.7° ; pulse, 118. 4 p.m., headache still severe ; pain and tenderness at epigastrium ; pain in loins and calves ; face a little flushed, but no marked facies or thoracic injection ; rather intense photophobia ; tongue, furred ; skin, hardly moist ; vomit, yellowish, with a good deal of mucus ; no ague parasites could be found in blood films ; temp., 37.6° ; pulse, 108.
 „ 13. Headache less ; skin moist ; no icterus ; no conjunctival injection ; no pain ; no vomit ; complains of weakness ; temp., 37°, pulse, 84. Evening, temp., 37°, pulse, 98.
 „ 14. Some headache ; no icterus ; tongue, slight fur, edges red ; papillae swollen ; tenderness and pain at epigastric angle ; no albumin (boil and nitric) ; temp., 37° ; pulse, 98.
 „ 15. No icterus ; no fever ; no albumin ; gums rather swollen.

Case 2. Live in chalet G. This chalet is very full of mosquitoes, partly *S. fasciata*, chiefly bred in the ant guards of the pillars supporting the house, and partly with *C. fatigans*, probably from the stables. Arrived in Parà from France, July 14, 1900. (Two other occupants of the house arrived in April; they were not attacked); sleeps with inefficient mosquito net.

- Sept. 15. Taken suddenly ill in evening; at 8 p.m., temp., 40°.
- „ 16. Face and eyes injected; skin, flushed; no headache or pains; no vomit; skin dry; tongue, central fur and very red edges; no icterus; no albumin; gums not swollen; slight injection of fauces.
- „ 17. Still flushed; hardly conscious; some albuminuria; no icterus; spleen and liver, nil.
- „ 18. Still flushed; temperature falling; fair quantity of albumin in urine; no icterus.
- „ 19. Feels better; no icterus; gums not swollen.
- „ 20. Some epistaxis; gums slightly swollen; (?) slight conjunctival icterus.
- „ 21. Some epistaxis; gums much swollen.
- „ 22. No epistaxis; bleeding now from gums.
- „ 23. Distinct conjunctival icterus; gums bleeding; albuminuria.
- „ 26. No icterus; feels weak.
- Oct. 2. No albumin; appetite returned.

Case 3. Middle aged, hearty but spare; cowkeeper, lives at stable; takes meals in chalet G. Arrived with wife from France and came straight to Institute.

- Oct. 1. Taken ill in the afternoon; temp., 39°.
- „ 2. Complains of frontal headache and lumbar pain; temperature normal; no sweating; examination of blood for ague parasites negative; spleen and liver, nil; eyes rather injected.
- „ 3. Eyes injected; epigastric pain and frontal headache; tongue, much moist white fur; no icterus; much bilious vomiting; no albuminuria.
- „ 4. Headache persists; no icterus; bilious vomiting, returns all ingesta; abdomen not tender; tongue, much white fur; acute epigastric pain relieved by vomiting; much albumin in urine; blood examination negative.
- „ 5. Less pain; abdomen rather tender; insomnia; much albumin; no vomiting; no icterus.
- „ 6. Pain slight; generally better; albumin moderate; no icterus; weak and emaciated. Further history: no icterus; no haemorrhage; gums, nil.

Intermediate between these two cases a labourer living at the further end of the grounds was taken with fever. After four days, when the effect of the quinine which he had taken had passed off, quartan parasites were found in his blood.

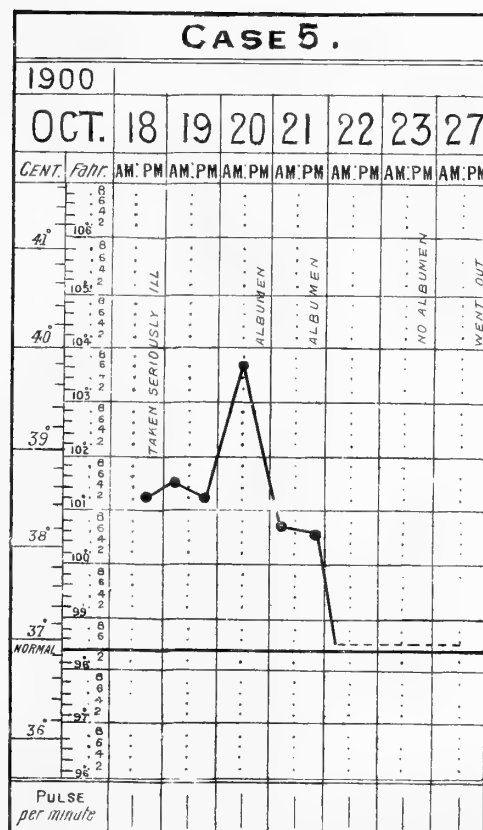
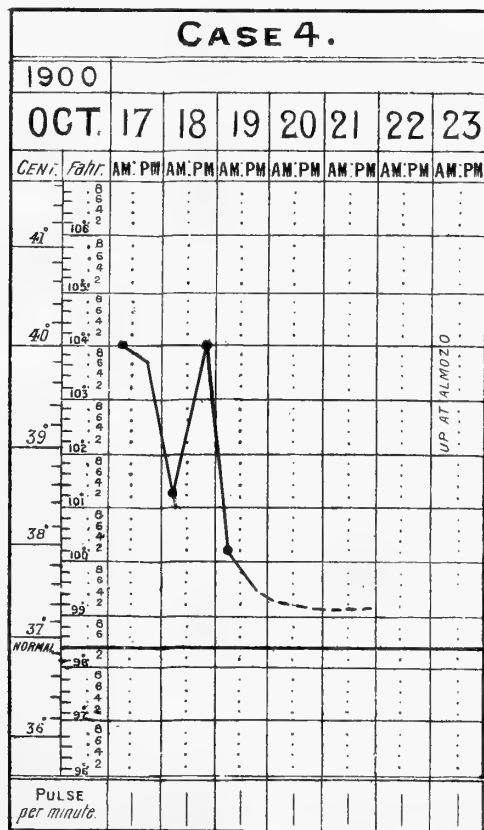
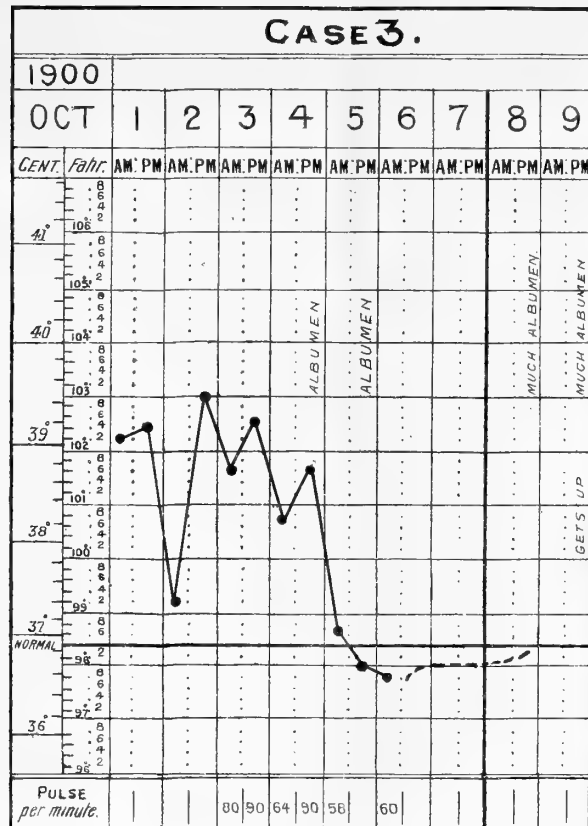
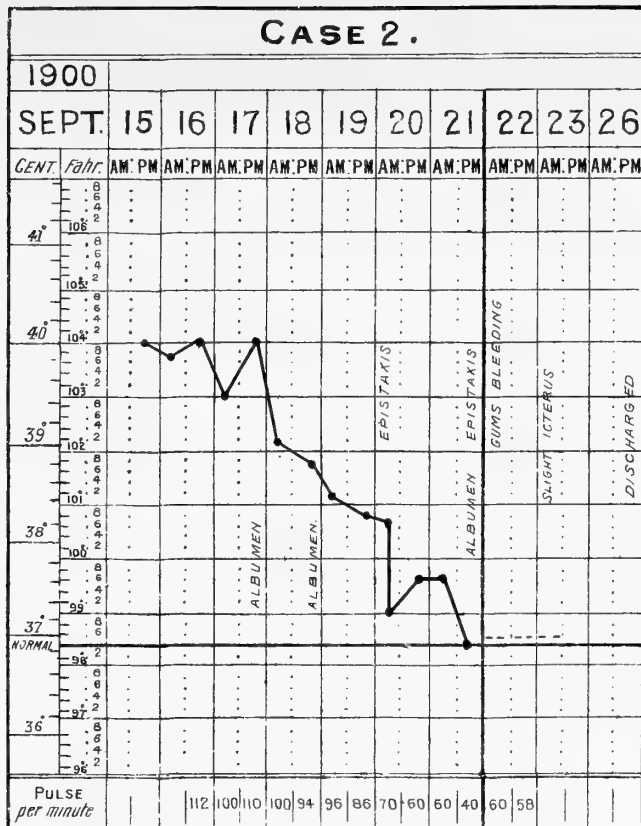
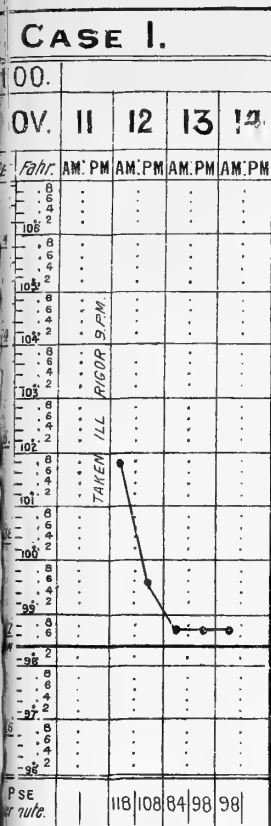
Case 4. Brazilian, has lived many years in Rio de Janeiro. Twelve months ago spent some months in Europe, on account of severe attack of fever (malaria). Has lately been paying a good deal of personal supervision about the cowsheds and stables.

- Oct. 17. Taken ill, the symptoms commencing in the morning ; the attack ascribed to going out the previous evening without a hat. Lay down for a while before dejeuner, at which meal ate nothing, as everything 'tasted nasty.' Soon after had a shivering fit, and about noon temperature was 40° , there was some sweating ; the temperature was taken at frequent intervals, and remained about 40° . At 6 p.m., temp., 39.7° ; pulse, 114 ; face, pale ; hot dry skin ; no pain ; no cough ; no vomit ; no diarrhoea ; on the left wrist were noted five neat mosquito bites (rather typical).
- „ 18. Morning temperature, 38.5° ; rather uneasy mentally. 5 p.m., temperature 40° , pulse, 100 ; no icterus or vomiting. On this as on the previous day we desired to test for albuminuria but, promises notwithstanding, we could not obtain a specimen.
- „ 19. The fever has abated to about 38° ; no icterus. Patient was weak and pulled down and returned to his ordinary occupation about the twenty-fourth.
(About ten days later—October 28—patient's wife was laid up for a few days, but probably no importance should be attached since this was the case intermittently, chiefly, apparently, on account of the climate, which generally does not cause European women to thrive.)

Case 5. Female Portuguese (25). Arrived in Parà, July 17, 1900, and has lived all the time at the Institute.

- Oct. 18. Taken suddenly ill ; much headache and photophobia ; bilious vomiting ; temperature, 38.5° . The temperature was above 38° for the next two days ; on the twentieth and twenty-first there was slight albuminuria ; on twenty-second temperature fell to normal. There was no icterus.

About this time, or a little later (dates and notes lost) another Portuguese (case 7) was ill with a passing attack of fever, of undetermined nature. Our Chalet was situated between that of the last patient, this patient, and the Chalet G. We watched the course of the attacks with some interest, as it might afford interesting data concerning the spread of yellow fever. It came out that in the month of July, 1900, an occupant of one of the chalets had had severe yellow fever, and had died during transference to the yellow fever hospital. Before discussing further, it may be stated that no *Anopheles* were discovered about the institute ; the nearest breeding-place that was found was three-quarters of a mile away. Except cases A and B, moreover, probably none of the cases were malarial. Case 5 is of interest from the malarial history, but I think that, probably, his illness was yellow fever of a typical character as occurring in an 'immune,' especially from the slowness of the pulse (*e.g.*, 114 when temperature was $39.7 = 103.4^{\circ}$ fahr., and again 100 when temperature was $40 = 104^{\circ}$ fahr.) ; there was no anaemia such as was observable in many other slight malarial attacks, nor were there subsequent fever attacks ; lastly, there was no actual intermittence of fever or typical ague attack during the illness, which lasted but a few days. With regard to Case 2 there can be no doubt that this was yellow fever, and



the diagnosis of Case 3 as yellow fever can also hardly be questioned. Case 4 has already been discussed; Case 5 was also, probably, mild yellow fever, and was diagnosed as such at the Hospital.

Although it is not clear where the original infection came from, and the records are imperfect, the mild case of fever (Case 1) is of interest, since this may have been the 'infecting case' for the husband (Case 3), with a twenty-day incubatory interval. The connexion between cases 1 and 2 probably must go back to the same source of infection. It is also of interest that, although the cases started in the chalets on the one side of ours and then appeared on the chalets on the other side, we ourselves did not become infected. It may be noted that we were generally away during the daytime, and therefore avoided the active time of the local *S. fasciata*, and at night were so efficiently protected by mosquito nets that we were hardly ever bitten at night by *C. fatigans* nor at dawn by *S. fasciata*.

Our own illness was so long after these cases that I do not think it can possibly have been directly derived from them. It happened that we projected altering our abode to the neighbourhood of the yellow fever hospital, chiefly owing to the rains and the deficient means of transport to and from the Institute. However, on January 10, we learned that one of the nursing sisters was laid up with yellow fever (from which happily she recovered), and about a week previously another sister had been laid up with a slight attack of fever 'which could not be yellow fever because she was a Brazilian.' We consequently judged that the hospital should be considered an infected house, and that it would be better not to transfer to its neighbourhood.

On the 14th we performed two autopsies (one on a yellow fever case and the other not); that day we remained to dine at the hospital, and returned to the Marco about 8.30 p.m., otherwise we had not been at the hospital after dark since December. Seeing that I was taken ill with a rigor and vomiting on the night of the 15th, I do not think it possible that we could have been infected owing to this exposure, for the incubation would have been very considerably less than forty-eight hours. Curiously enough, just four days before, I had been most of the day catching mosquitoes about the sewage outfall of the hospital; but as my companion had never been near the place, and was almost certainly infected simultaneously with myself, the infection cannot have been due to some bite received there. Most probably we must have been infected whilst about the wards of the hospital, or whilst at lunch some days before; if this is the case, the infection must have been during the daytime, and therefore by *S. fasciata*. It remains to be said that my colleague was taken suddenly ill on the morning of the 16th of January, and unhappily he went rapidly down hill, and anuria setting in, carried him off upon the 20th, notwithstanding the constant care of the GOVERNOR, Dr. PAES DE CARVALHO, Dr. PONTES DE CARVALHO, and the assiduous ministrations of the nursing staff.

F. YELLOW FEVER ON SHIPS

S.S. 'D.' Arrived at Pará on November 25. Perfectly good health on voyage ; none of the crew allowed on shore ; the only things brought on board from shore were beef, fish, vegetables, and ice ; all of this carried out from the city (about two miles away) by the tender ; otherwise only discharge of the cargo has taken place, namely, into nine lighters. No water was taken on board.

On December 2, B. (third officer) taken ill ; seen on eighth day ; certain yellow fever ; transferred to hospital.

On December 7, boy (mess steward) taken suddenly ill, with temperature of 104.5° .

On December 8, ordinary seaman taken ill with fever.

On December 10, second officer was not well, but no rise of temperature.

Owing to only receiving information concerning this just before the vessel sailed, these last three cases could not be examined ; it was stated that none had any jaundice, nor did they have definite sweating attacks.

The vessel then proceeded to Manáos to discharge and receive cargo. It was stated that on the voyage up river one ordinary seaman was taken ill, but no details were forthcoming.

On leaving Manáos, D.I., a fireman, was taken ill on December 19. He was transferred to hospital on fourth day, where he showed symptoms of severe yellow fever (black vomit, bleeding from gums, delirium, icterus, prostration, and large quantities of albumin in the urine).

S.S. 'H.' Two men (third steward and a fireman) were taken ill on leaving Manáos, where the vessel had been for twelve days, on February 24, 1901. The former had a well marked moderately severe attack of yellow fever ; the other gave a history of bilious vomiting, but on admission to hospital, on March 1, no definite symptoms of fever could be detected.

In the case of the steamer 'D,' it is certain that at least one case of yellow fever developed on board in a person who had not been within about two miles of the city (Pará). The only possible modes of convection of the infection would be (1) the food brought off, (2) the contact with the labourers who came to discharge the cargo, (3) infective material brought off in the tender or lighters, other than the items mentioned in (1) and (2).

The first two points may be discarded, for it is unlikely that so few out of a population of thirty should have been taken ill if infected food had been the cause ; in regard to the second heading, we know that far more intimate contact (*e.g.*, handling yellow fever patients or performing autopsies) than would occur under the conditions is not able to confer the fever.

In considering the last heading, the most striking objective feature is the transportation of gnats. All ship's captains are agreed that gnats come on board with the advent of the lighters, and the men on the tenders complain of the abundance at night when lying close to the wharfs. The species found on lighters were 'town' kinds—*S. fasciata* and *C. fatigans*.

It may be mentioned here that during my trip up the river to Manáos, all gnats which we caught (with the kind assistance of the officers and a fellow-passenger) were preserved day by day, and *S. fasciata* did not occur until the day after arrival off Manáos, when ten specimens were taken, presumably they came off the lighters. Only other species came on board during the passage.

Investigation of a number of lighters at Pará shewed that *S. fasciata* larvae were present here and there on the bilge of the covered lighters, unfortunately they had been recently cleaned out and painted, so that probably the larvae were unusually scanty. The open lighters, some of which contained a considerable amount of water, were free from larvae; this was ascribed to the fact that they are used for coal (also salt, etc.), and the contained water was covered by a thin film of tarry or oily matter. The adult insects, however, would have to become infected; if this could occur, from feeding on the labourers more widely spread and more frequent outbreaks would be probable on these ships. It seems more likely that occasional infected gnats might be carried from the city in the lighters or steam tenders to the ships. The local conditions are such that the mode of transfer of yellow fever by gnats, is highly probable.

Several cases occurred amongst the persons who brought out steamers for the river service; here, however, the vessels lay close up to the city, and the individuals in question were in the habit of spending time on shore, so that no satisfactory evidence is afforded of the mode of infection. In all these cases pertaining to ships it is difficult to get satisfactory accounts, and, moreover, by the time that reports are received too much delay has intervened.

Nothing very definite is afforded by the following instance of a sailing vessel from the point of view of mode of transfer of the fever, but it shews how severely a vessel may be attacked about Pará:—

The barque 'C.P.' arrived off Pará on December 22, 1900, from Antwerp, laden with rails and other cargo, such as cement, under the German flag. The captain and crew consisted of fourteen persons all told. The vessel lay off the city abreast of it, that is, a matter of a few hundred yards distant, until February 4, when she was taken alongside the wharf to load with ballast for a week. This and other information was kindly given me by the captain. Mosquitoes did not appear on board till about a fortnight after arrival, *i.e.*, about 1st of January. (When I visited the ship they were appallingly abundant—*S. fasciata* and *C. fatigans*). Crew only went on shore with boat to return at once, and were never on shore at night.

Case	When taken ill	When admitted to Hospital	Result	Diagnosis
	(Arrival at Para, 22. xii. 00)			
1—V.F.	12. i. 01	16. i. 01	+ 16. i. 01	Yellow Fever
	(Discharge of cargo into lighters began 15. i. 01)			
2—J.B.	15. i. 01	17. i. 01	Recovered	Yellow Fever
3—A.F.	18. i. 01	21. i. 01	Recovered	Yellow Fever
4—E.	18. i. 01 ?	18. i. 01	+ 22. i. 01	Yellow Fever
5—M.	19. i. 01	21. i. 01	+ 23. i. 01	Yellow Fever
	(Discharge of cargo finished 22. i. 01)			
6—T.	26. i. 01	31. i. 01	+ 1. ii. 01	Yellow Fever
7—D.	28. i. 01	31. i. 01	Recovered	Yellow Fever
8—K.	3. ii. 01	6. ii. 01	+ 8. ii. 01	Yellow Fever
	(Went alongside wharf 4. ii. 01)			
9—W.	6. ii. 01	9. ii. 01	+ 12. ii. 01	Yellow Fever
	(Returned to mooring about 11. ii. 01)			
10—P.	18. ii. 01	21. ii. 01	Recovered	Yellow Fever
11—C.P.	23. ii. 01	25. ii. 01	Recovered	Tertian malaria and ?
12—S.M.	24. ii. 01	25. ii. 01	Recovered	Yellow Fever

The captain had an attack of yellow fever four years previously at Rio de Janeiro. The other hand (not included above) had also had yellow fever some years before. Most of the above cases occurred during my own illness; they were diagnosed as yellow fever at the hospital, and such notes as were made and the temperature charts agree with this. The last three cases noted (namely, 10, 11, and 12) were examined; both Nos. 10 and 12 were indubitable yellow fever, rather severe in type. No. 11, however, certainly had tertian ague, for the parasite was demonstrated in his blood; he neither had jaundice nor albuminuria. His personal history was that he had had yellow fever at Santa Cruz six years ago, and again four years ago; he denied having had ague, or that he had been at all ill during the last four years when his voyages were between Hamburg and North America. It is possible that the virulent infections which were occurring of yellow fever organisms may have roused up a latent old malaria infection, although his immunity was sufficient to prevent any marked sign of yellow fever. None of the rest of the crew showed indications of an ague infection.

S.S. 'A M.' arrived at Pará, October 8, 1900. The vessel lay close to the wharf, on the mud at low tide ; the proximity to the city and the fact that the men went on shore prevents any direct information. The chief engineer died of an 'access of pernicious fever ;' this occurred after about six or seven days of illness, and patient was very jaundiced at death as I was subsequently informed. The boatswain had a well marked severe attack of yellow fever, and a week later the third engineer was taken ill and died also of well marked yellow fever ; it may be remarked that the diagnosis of 'pernicious access' is a not uncommon means of reducing the yellow fever mortality and increasing the apparent danger of the city.

Whilst isolated cases recognized as yellow fever occurred occasionally on the ships which do not approach close to the cities (Pará and Manáos), occasionally cases of slight fever occur, the nature of which is not clear from the accounts which can be obtained of them. One case, which was seen a week after being taken ill, may be cited. S.S. 'L.,' steward taken suddenly ill with a rigor, the thermometer shewing 103° F. ; he was treated with a purge and quinine ; the next day there was still fever, and the hands and up to the elbows were described to have become swollen, 'like dropsy ;' on the third day this swelling went down, and patient was feeling much better, but very weak ; at the end of a week the only complaint is of weakness ; there are no objective symptoms, *e.g.*, icterus (unfortunately there was no means of testing the urine for albumin), except that the right axillary glands were palpably enlarged, and there were signs of many old bites about the hands.

VII. AGUE AT PARÀ AND HABITS OF *S. FASCIATA* AND *C. FATIGANS*

The species of *Anopheles* met with about the Amazon is one with white tarsi to its hind feet, the extreme tip of the foot being black ; it is identified as *A. argyritarsis*.

It was intended to experiment with these in order to ascertain whether it was a favourable host for the gnat cycle of the ague parasites. Except at the commencement of our stay at Parà, it was difficult to obtain larvae or pupae in abundance, and at the time named we were too much occupied with yellow fever. Had we anticipated this difficulty it would have been well worth while to have sacrificed some time to the matter whilst the supply was abundant. It is, therefore, only possible to state that the houses near the breeding places where this species was found were all ague stricken ; in some cases the presence of the ague parasite was determined in the blood of persons from such houses.

Two individuals of another species (*Anopheles lutzii* Theo.) were taken ; this has unspotted wings and is all dark, with the exception of a dorsal longitudinal white line. None of the larvae from different pools hatched into this kind ; its breeding places were not found.

The larvae of *A. argyritarsis* were found in small muddy pools a few inches deep, the largest pool was only about six feet by three feet. They were never met with in large or deep collections of water in pools containing obvious green algal growth. They are extremely active, and on the slightest disturbance seek refuge in the mud, so that it is possible to bale out much water with a small cup without catching a single individual unless some of the mud is scooped up. It was found in practice that a gauze net was the best means of catching them, the individuals being picked out with a wire gauze spoon ; in this way one could avoid carrying much mud and predacious enemies, such as tadpoles and dragon-fly larvae. In all cases where *Anopheles* larvae were found they could be detected by inspection for a few minutes ; I never succeeded in catching any with the net in pools in which they could not be detected by watching.

Observations on some pools in the proximity of some brickworks teach the lesson that the absence of *Anopheles* larvae in given pools may be temporary, and indeed, follow an extraordinary abundance.

Four pools in some sand diggings, close to some brickworks, were examined on many occasions. There were cases of 'seisoës' in nearly all the huts in the neighbourhood ; two huts had all the occupants sick except one individual ; one of these

huts was occupied by new-comers, (man, wife, and three children). Three of these were examined, and certainly were suffering from malaria, and the other two were also ill.

Pool 1. (About fifteen by eight feet), muddy, moderate amount of green algae ; one end shallower and eventually cut off during drier weather ; as seen when nearly dried out, probably deepest part when full not much more than a foot. Interval between Pools 1 and 2 = twenty yards.

Pool 2. (About thirty by fifteen feet), deep; very much green ; never dried out; water rather muddy. Interval between 2 and 3 = fifty yards.

Pool 3. (About six by four feet), shallow, not more than about 6-8 inches ; muddy ; no green growth. Interval between 3 and 4 = sixty yards.

Pool 4. (About twenty by twenty feet), rather muddy generally; red growth, sometimes intensely ruddy ; no green obvious more than one foot deep.

Pools 2 and 4, never yielded any *Anopheles* larvae.

When first examined about the middle of September, Pool 3 was thickly crowded with *Anopheles* larvae (= + + + +), none were found in the other pools.

Sept. 29. Pool 3. = + + + + Pool 1, -0.

Oct. 4. „ 3. = Very scanty ; many water beetles.

„ 10-16. „ 3. = Very scanty ; pool drying up.

„ 19. „ Extremely scanty ; pool much reduced.

„ 23. „ 3. Dry ; other pools much reduced.

Sept. 1. Pool 1. Few + especially at end cut off from general pool.

„ 2. „ 1. As yesterday ; Pool 3 consists of a few hoof-mark puddles = 0 ; Pool 2, no *Anopheles*.

„ 12, 13, 15 „ No *Anopheles*.

Dec. 3. Pool 1. Few *Anopheles*.

„ 7. „ 1. No *Anopheles*.

It may be noted that these pools were much haunted by dragon flies, and there were many water beetles, and a 'white and dark' water boatman, the presence of which may account for some of the diminution or disappearance of the larvae ; *Culex* larvae were also present (= *Taeniorhynchus fasciolatus*).

The following localities shewed the presence of *Anopheles* larvae :—

At Parà. 1. Shallow muddy pools about brickworks near Travessa Bonifacio, already mentioned.

2. Shallow muddy natural pool (about six by three feet) below lower corner of Baptista Campos. Larvae scarce; a case of tertian fever was examined which came from a house not far distant.
 3. Tiny natural puddles at the S. Jronymo end of Trav. 3 Maio; some of the occupants of the surrounding houses stated that there was 'much fever,' but no cases were actually examined; search for adults in some of the houses not successful.
 4. Wheel track puddles of muddy water near house of Senor M., beyond Marco da Legua. A man was taken ill, in a hut a few yards away from the puddles, and shewed tertian parasites in his blood.
- At Mandos.* 5. Wheel track and natural puddles at Cachoeirinha, Manáos; all shallow and muddy, without green growth, but extending here and there into the grass (rainy season). This region has the reputation of being the ague locality in Manáos: it consists of a comparatively high-lying plateau away from 'swamps,' and, in a general way, does not look like a fever stricken place. On this plateau of a few acres extent, large numbers of *Anopheles argyritarsis* larvae and pupae were readily collected. One point of interest is that a few individual larvae were found in the pools on the course of what becomes a miniature torrent during a rain shower, and carries the water down to the igarapé (stream) some fifty or sixty feet below; from the steepness of the descent, and the small size of the puddles, and the direct evidence of drainage from *Anopheles* puddles on the edge of the plateau, it is most likely that these isolated single larvae were carried down from above.

From what has been said it appears that this species favours small very shallow pools; these consisting of opaque muddy water, and freedom from growth makes the larva a very conspicuous object to inspection. The small size of the pools makes them eminently suitable for filling in or for treatment with kerosene, etc., and whenever pools or collections of water were met with in or about the city they were always inspected, and often netted also, but the above-mentioned places alone yielded larvae; pools that were only observed once may have been temporarily in abeyance.

Dr. FURNESS, of Bahia, told me that he had met with the larvae (of the same species) in the 'ant-guards' in gardens. In Pará I did not meet with any in these water collections, although in one garden adult insects were met with and a natural breeding place was found not many hundreds of yards away (*B. campos*). Nor, again, were larvae discovered in swampy and overflowed districts in the forest and outskirts of the city. During the prolonged operations for laying a fresh tramway track there was much water lying about in the Estrada da Independencia for months, but these pools were not affected by *Anopheles*.

The adult insect as seen in 'bred' specimens is very sleepy during the daytime, it sits on surfaces in the 'correct' attitude, the abdomen being inclined at an angle of about 45° to the vertical. The hind legs and tarsi are generally kept close together, contrasting with the widely separated hind legs of the 'culices.' In two houses (one on the *Est Nazareth* and the other on the *B. campos*) adults were seen on the wing after dark, about 7.30 p.m. To my knowledge, I was only bitten by *Anopheles* on two occasions, once about an hour before sunset (about 5 p.m.), and again about an hour after sunrise (about 7 a.m.); there was no untoward effect. This is simply noted as evidence that this species may bite during daylight, at any rate at the beginning and close of the day.

By local repute there is much malaria at Parà; but out of the Englishmen and other foreigners that I met living in different parts of the city there was only one who had definite ague attacks, and he was in the habit of going out on expeditions, and did not trouble about taking a mosquito net. The fringe of the city bordering on the swampy low-lying regions are responsible for most of the cases. By reputation the more out-lying district, where the huts are right in the swamp, are bad for ague, but in general the dwellers, who were questioned in these parts, where the huts are some distance apart, denied that they had fever, and dredging and inspection of the overflowed ground failed to yield any *Anopheles* larvae.

Blood examination of cases about the city generally shewed tertian parasites; one case of quartan was seen. Crescents and so-called aestivo-autumnal parasites were more common amongst the cases coming from the islands; these persons were often in a most dreadful condition of extreme anaemia, their appearance quite waxy and almost translucent, their blood extremely watery, and shewing marked alterations of their few red blood corpuscles (macrocytes, microcytes, poikilocytes, and nucleated reds).

Positive diagnosis of ague by blood examination is by no means easy in the semi-civilized population of Parà. This, I think, is attributable to the wholesale faith in and use of 'Remedios,' and a case of illness of any kind which has not had quinine in some form or other is a rarity. The importance of distinguishing mild cases of yellow fever from ague was not appreciated, and notwithstanding oft-repeated requests to withhold quinine, often even in cases in which there did not appear to be any indication far less any necessity for the drug,* we generally had to be content with a negative examination, which naturally left the diagnosis uncertain. Diagnoses of malaria too help to lessen the yellow fever reputation of the city.

In some of the rubber-cutting districts in the swampy riverside forest there is much malarial fever. Arrangements were to have been made for us to visit some

* Thus one has seen quinine given to perfectly afebrile cases.

bad localities so that practical suggestions might be made to deal with the local conditions. However, this visit was put off. Unfortunately, however, no arrangements were actually made, and, therefore, no suggestions can be made. By repute several places which were very fatal to early pioneers are now regarded as comparatively healthy; even if this be true the possibilities are too numerous and vague to make discussion profitable.

Elsewhere the question of the clinical condition of the superficial lymphatic glands is raised, and it may be repeated here, since if enlargement does not occur, for instance, in the axillary glands in malarial, but not yellow fever districts, the point may be of some use in the diagnosis of yellow fever, especially in mild cases, and perhaps also in malarial individuals.

It may be of interest to mention that quite a number of Brazilians (medical and lay) hold that malarial attacks are apt to occur at monthly intervals, some even go so far as to consider that the moon is the directive force.

Some use was made of the centrifuge to assist in the discovery of malarial parasites. The scheme was to take about three volumes of blood into a capillary (such as one ordinarily uses for sedimentation tests) containing about one volume of citrated salt solution (one per cent. citrate of soda giving eventually one-quarter per cent., which prevents coagulation); after mixing well, the tube is sealed at the end, and centrifugalized for a few minutes. Three layers are obtained, supernatant plasma (with many suspended platelets), protoplasmic layer (containing leucocytes, platelets, and crescents), if present in the blood, red corpuscle layer. In a warm climate, if the tube is kept some time before it is centrifugalized, the leucocytes may have crawled about on the glass, and thus make an unduly thin layer above the red. By cutting off the tube just above the leucocyte layer, the leucocytes and upper layers of red layer may be removed with fine capillary and examined. For making permanent preparations, it is best to fix rapidly, as by placing the films at once into a petri dish into which a drop of strong formalin has been put; red corpuscles are apt to crenate more rapidly from the diluted plasma than from plain blood. The leucocyte layer forms an easy means of detecting pigmented individuals, and thus assisting diagnosis, and also gives concentrated specimens where it is desired to make differential counts of the varieties of leucocytes. We also tried using a fixing fluid for the dilution, such as saline solution, with a trace of corrosive sublimate, naturally the proportion of blood taken must be less, otherwise clotting will occur. •

Yellow Fever and Ague. It has sometimes been stated that there is some antagonism between the two diseases. Out of our seventeen autopsies, in four cases there were malarial spleens; one of these is interesting, and is in accordance with the chances of infection. The patient was a Bolivian soldier who got malaria up river on his passage over from his home country; a few days after arrival in Pará he contracted fatal yellow fever. So far as Pará is concerned, an immigrant coming

into the city is likely to have yellow fever in mild or severe form first, so that by the time he gets malaria he has already acquired yellow fever immunity. There does not seem to be any indication of a mutual protecting power between the two diseases.

Stegomyia fasciata

Distribution. Common house mosquito about city and outskirts, as at yellow fever hospital, Hospital Santa casa, Marco da legua, Asilo dos alienados (Leper-asylum), also at Pinheiro (ten miles down river from Parà), and Outeiro. Also on vessels and lighters lying off Parà. At Manáos, in many houses about city and suburban region 'Cachoeirinha.' Never seen out in forest away from houses, or in isolated huts situated away in forest. Not seen at Santa Anna, some twenty-five miles north of Parà, or Fazenda Natal, in Marajo.

Breeding Places. Casual waters in vessels, etc., in and about houses such as buckets, tins, washtubs, rain gutters, ant-guards (perforated troughs to protect plants in gardens, and sugar, etc. in houses), larger and deeper collections of water as casks or hogsheads full of rain water. Also in bilge water of barges, lighters, and s.s. Viking (Amazon Telegraph Co's ship, many years on the river). Not found in sewage collections as cesspools, stable runnings, etc., although found in neighbourhood in cleaner waters. Also not found in natural puddles in forest or streets, etc.

Habits of Adult. This species in Parà is solely a day gnat. It consequently afforded means of observation. It is on the wing and will bite shortly after sunrise, (about six a.m.); again, a few have been observed biting about eight to nine a.m., after which there is a pause till about eleven a.m., when again a few may be feeding. The time of chief activity is in the middle of the day, from about twelve to two p.m., they then bite freely, and are seen to copulate on the wing in numbers; another pause follows, though there may be a few about, but they do not cause trouble when one is sitting at the microscope until about half-past three till about five p.m. After dusk or dark I have only once met with a specimen; this was a male, feeding in a sugar-basin rather before seven p.m. These statements are derived from observations whilst working in the laboratory, and during a residence for a week in the house of a gentleman, whose garden was liberally supplied with ant-guards (perforated troughs filled with water, for preventing the access of the destructive 'Saüba' to the plants), each of which was full of developing larvae and pupae. Sitting in the verandah of this house it was easy to catch fifty to eighty specimens without moving from one's chair, in the early hours of the afternoon, yet after sun-down, not a single individual was met with.

In so far as yellow fever infection is associated with this insect, it is of importance to note this in relation to the common idea that the fever is commonly caught at night. It would appear that night would only be dangerous from this insect in so

far as the first of the day after sunrise is included in the term 'night.' In this connexion it is of interest to note that all the artificial gnat infections carried out by the American commission were done during the day-time, *S. fasciata* being the species employed.* Besides feeding on man, I have observed it on dogs, on an agouti, and a bat.

The periodicity of feeding activity was more closely observed on this species, but I rather believe that a similar intermittence of rest and activity occurs with other gnats, 'night' as well as 'day' ones.

One batch of *S. fasciata* was put with a number of living butterflies, but they did not feed upon them; on the assumption that night-feeding insects attack night-sleeping animals, and *vice versa*, an experiment with moths would have been more apt, but none were available at the time. It may be mentioned that the period of greatest activity of *S. fasciata* corresponds with the time of siesta of the human being.

On one occasion embryo filaria was found in this species; the source of its infection was not found, but the suggestion is that it would have been a diurnal worm.

The eggs are not laid in an adherent raft; they are practically separate, and are deposited close to the edge of the water, if not sometimes actually on the surface of the containing basin. At any rate, they readily adhere to this surface sufficiently firmly to prevent detachment by a stream of water; this is probably of use where the breeding place (*e.g.*, a small tin or a roof gutter) is exposed to tropical rain.

The larva is easy to recognize from its curious opaque appearance; unfortunately no description was made. As has been observed by Dr. FINLAY, of Habana, as regards spontaneous drying† I have seen almost full-grown larvae appear very shortly after putting water in a 'dried' basin in which development had been occurring. In two cases the time of cycle from laying of egg to imago was noted; in one the first pupa appeared on the eighth, and hatched into a male on the tenth day; in the other, the first hatch occurred on the twelfth day; in both cases larvae continued to be present for weeks after the first hatch. (It need hardly be mentioned that the vessel was effectively screened against intrusion of other possible egg layers).

The adult insect varies considerably in size; this is true of both sexes. When sitting on a vertical wall the abdomen is tilted towards the wall, so that the tail nearly touches it (*i.e.*, in the contrary sense to the orthodox *Anopheles* posture). The hind legs are separate from one another, and away from the body the tarsus curves downwards, but tends to a more or less horizontal position.

* It is, perhaps, unsafe to dogmatise absolutely that any gnat is purely a 'night' or purely a 'day' biter.

† N.B.—The atmospheric humidity in Para is never very far from the saturation point.

Culex fatigans. WIED

Distribution. This is the common house mosquito of the city, in this respect resembling *S. fasciata*, but differing in being a night insect; although found in and about the city and on lighters lying in the river. It was not very common about the Hospital Domingos Freire (yellow fever), and about the first occasion it was met with there was coincident with the occurrence of a spontaneous yellow fever case; *S. fasciata*, on the other hand, was abundant there. It was not met with in huts away in the forest; nor did it come on board the steamers while going up the river.

Breeding Places. This species seems especially to affect foul waters; larvae were found abundant in stagnant pools of stable runnings, and also in a cesspool, the covering of which was damaged. Larvae were not met with in puddles in the forest. All attempts to breed it from the egg in clean water in the laboratory resulted in death of the larvae a few days after hatching. In fact, it seemed to be essentially a 'filth' loving insect.

Habits of Adult. This species seems to be a purely night insect; often a crowd of males would appear just after dusk in our chalet. Numbers of females full of blood were easily obtainable in houses in the city. The eggs are laid in a raft of vertically disposed black eggs.

VIII. GENERAL HEALTH OF PARÀ

The city of Parà, or Belem do Parà, is situated slightly south of the equator (lat. $1^{\circ} 26' 59''$ S., and long. $48^{\circ} 30' 0''$ W.), and about seventy miles from the ocean. It is built on a tongue of slightly raised land, which forms a sort of flat ridge, at the end of which is the river front (Rio Guajara), and on either side are swampy, low-lying districts, into which the fringe of the city extends. The elevation, though very inconsiderable, is ample to give a very fair gradient for drainage; at the same time the soil consists of sand, and through this much of the fallen rain water soaks away, where from traffic, etc., there is no coating of mud. The tide helps to cleanse the river front, the rise at spring being about ten feet; but the wharfside bays are sufficiently sheltered from the current to allow the accumulation of much filth, in places a bed of mud being exposed at the fall of tide.

The general health and mortality in the city must be looked at from two stand-points, namely, that of the native and old resident and that of the newcomer. For the newcomer, as in other regions where yellow fever is endemic, is liable to undergo risks of becoming infected with this disease and to die of it; consequently, especially in times of considerable immigration of susceptible persons, the apparent death-rate is not a fair estimate of the mortality due to all the local conditions which affect the well-being of those who have resided long. There are no figures to show the extent of the yellow fever risk in Parà; but it is to be seen from the cases, with which we had to deal, that the city is extensively riddled with the disease, for we had cases from houses in all quarters, even out to the Marco da Legua ('a league from the city'). So that there was no part (at any rate during the later months of 1900, when the considerable outbreak of the earlier months was on the wane) which one could confidently state was free from risk. Many of the milder cases pass unrecognized, and perhaps not a few of the more severe pass under other names; the tendency in yellow fever districts is, for those in control, to endeavour to make as little as possible of the disease, 'so as to avoid giving the place a bad name.' Naturally nothing could better tend to foster the disease than such measures. The incidence of the fever on a given number of traceable individuals cannot always be given, but figures are extant in the case of an Italian dramatic company, which visited Parà in April, 1900, and shew that out of the company at least twenty-nine individuals became infected, and at least nineteen succumbed to the fever; these include not only the lower grade of artists, but also some of the more reputable.

Considering the situation of the city so near the equator, the climate is less hot than might be imagined; the almost daily shower of rain, be it 'dry' or be it 'wet' season, conduces to produce this result. But at the same time it gives rise to the

excessive humidity of the atmosphere which is so enervating to the northerner. Local authorities love to quote the words of travellers who speak of the delightful freshness and coolness of Parà after being in the hot steamy interior, but they do not draw attention to the still more delightful freshness which is experienced in getting out to sea. The steamy heat reacts unfavourably on the energy of the native and foreigner, and makes the place rather unsuitable for the prolonged, continued residence of Europeans; so much so, indeed, that one of the leading physicians informed me that he always recommended foreigners coming from cooler climes to spend six months in Europe at least every three years, that is to say, a much shorter period than is customary in India and other tropical places. By heresay evidence I gather that some of the more successful business has been conducted by firms consisting of a number of responsible partners, each of whom is thereby able to spend comparatively short stays in Parà, and much time in Europe. Thereby a certain briskness and energy can be brought to bear in a manner which can hardly be expected from those who have made prolonged stays. So far as I was able to observe, the women from northern climes are particularly unable to withstand the climate; thus the wives of several residents have found themselves obliged to reside in Europe.

The general tendency to apathy and slackness is also due to the monotony of the conditions—uniformity of temperature year in and year out, no relief by change of season, except, perhaps, for the worse, during the so-called winter or wet season (January to May), when the humidity becomes still more trying, and the absence of recreation, so far as the majority are concerned. The business of one day ushers in the business of the next; and, on the whole, there is but little social intercourse. One difficulty in the way of obtaining recreation, which cannot be neglected in considering the opportunities for all, is the financial aspect. The price of things in Parà border on the absurd, especially when the rate of exchange is high; this makes itself felt, especially on those who receive a salary in sterling currency based on a certain rate, for instance, ninepence per milreis, a serious deficiency in the purchasing power of the salary, when the milreis goes up to twelpence or thirteence per milreis, as was the case during my stay.

Some break or change can be obtained by residing out of the city, as for example, further down the river at Pinheiro (one hour) or Mosqueiro (two hours); but the service of steamers is slight, and arranged more for government office hours, which are not found particularly convenient for business purposes. On the land side, where the air is not so fresh and breezy (as especially about Mosqueiro), many people live out at or towards the Marco da Legua (the mark of the league distance from the city), to and from which they travel by an erratic and miserable tramcar service.

The action of the climate upon Europeans is probably enhanced by the performance of brainwork under high pressure, now and again with unexpected worry for

having to deal with childish interferences in the way of rules and regulations, formulated from time to time by the authorities. The hours of business are long, usually about eight or nine a.m. to four or five p.m., with a break for 'breakfast' about mid-day. Frequently for the despatch of mails, the heads or principals return to the office again in the evening till a late hour. When the mail happens to be despatched on a Sunday the opportunity for a week end out of town becomes impossible. One week follows another, year in and year out, much in the same way, until leave of absence comes round. There does not appear to be any general rule of any sort of annual holiday for a few weeks as in cool climes.

With sufficient wealth and independence, conditions of life are naturally much better ; also at the other extreme, the labouring classes are better off, for wages are sufficiently high, and labour sufficiently scarce to make them fairly independent ; moreover, they are not troubled with the wear and tear of exchange, the price of rubber and the like ; and they are inured to the climate. The better class Brazilians in general are not particularly energetic, and many of them endeavour to make visits to Europe and the north for a change.

Diet. The food arrangements cannot be highly extolled, the chief difficulty being the scarcity and expensiveness of fresh vegetables. Beef forms the chief staple, and is raised on ranches in the neighbouring island of Marajo ; a better quality is now and again brought up by cattle boats from the Rio Plata, whence also occasionally some mutton is brought. Fish abounds in the rivers and many kinds are delicate for the table, but there is no proper organized scheme of transport, so that it is liable to suffer before or soon after it reaches the market ; I understand that an endeavour was made to start a reasonably conducted system of transport, but this was quashed by petty regulations. Fowls, ducks ('Muscovy ducks'), guineafowl, turkeys, etc., are also obtainable. Native animals, Páca gutia (agouti), tartaruga (water tortoises or 'turtle'), etc., are occasionally met with. A poor quality of bread is made from imported flour. Numerous kinds of vegetables for cooking or for salads are cultivable or native, such as sweet potatoes, mandioca, beans, pumpkins, eggplant, mascisce (a small kind of cucumber), carurú (often used as a crisp salad), vineigreira (sorrel like), lettuce, etc ; but the importation of potatoes, cabbage, onions, carrots, etc., from Portugal or the United States, saves the trouble of growing things locally. Some attempt has been made to introduce the cultivation of various northern plants, but seemingly no endeavour to improve or enlarge the supply of native products or of plants known to thrive in the climate has been made.

Of fruit also there is a considerable variety ; besides bananas, oranges, and pine-apples, which ripen all the year round, and the avacate (alligator pear) and mango, which have seasons, there are a number of native fruit, many of which are peculiar to the region. Cupuassú, Bácury, Genipapa, Sapotilha, Abiu (Sapotaceae), Ata Biribá (Anonaceae), Caju (Anacardium), Maracujá (passion flower), may be mentioned, some

being eaten fresh, others cooked or made into fruit drinks with the addition of water and sugar. Mention also must be made of the essentially Amazonian 'Assahy,' made from the fruit of the palm of the same name, by rubbing it with water and straining the resulting thick purplish emulsion, with a fascinating taste, which is taken with sugar and mandioca (cassava) meal; a similar 'drink' is made from the bacaba palm but has not the same reputation for delicacy of flavour.

The common light wines of Portugal form a common addition to the meals. Abuse of alcoholic beverages (*e.g.*, whiskey) by Europeans is happily not a prominent feature of life in Parà.

The poorer natives and Portuguese are, in general, not given to great varieties of diet; besides the staple Farinha (mandioca or cassava meal) imported dried salt codfish, imported rice, and imported dried beef (*xarque*), and dried beans, form the principle items. A large fish (*pirarucu*) is caught and dried and salted and used to a considerable extent, but is said to be less economical than the imported fish. The native alcoholic beverage is the spirit distilled from fermented juice of the sugar cane (*caixaça* or *cachaça*), and leads to a certain amount of intemperance.

Temperature. The temperature at Parà varies between a maximum of about 32° C to about 20° C (or in Fahrenheit scale, say 89·6° to 68°); during the five years 1896 to 1900* the highest recorded temperature was 33·5° or 92·3° F. During our stay we found the variation was between a day maximum of about 31°, rarely higher, occurring in the early hours of the afternoon, and a minimum of 21° at night during the 'dry season,' and 22·5° during the wet. The absence of really high degrees is probably due to the rain which falls almost every day in the afternoon during the so-called dry season, and the obscurance of the sun by the heavy rain or thunder-clouds. The rain showers are usually very localized, so that one day one part of the town or suburbs may escape, whilst the following day it may be the site of a heavy drenching; consequently the observations with a single rain gauge give but slight ideas of the general rain fall in the area. During the dry season (June to December) rain seems rarely to fall at night or in the morning; there is a very heavy dew, so that in the morning grass, shrubs, etc., are soaking wet.

The humidity of the air arising from the evaporation of immediately fallen rain, the neighbouring swamps, rivers, and backwater, is very considerable, and generally approaches the saturation point. The atmosphere is often particularly stifling about five to seven p.m., when the air is quite calm. The afternoon rain is ushered in with a squall of wind.

From what has been said it may be gathered that it is rather the brainwork, combined with a considerable amount of monotony of climate and employment in an enervating climate, without much chance of recreation, that tells upon the health

* *Parà Medico*, I, No. 4, p. 112.

and vigour of Europeans, and makes comparatively frequent sojourns in more temperate climates advisable. With solely the uneventful and unworried mode of life in the forest, away from the cares and anxieties of commerce and civilization, the conditions are probably more favourable for prolonged residence in the Amazon valley. Thus, as an isolated instance, I may mention meeting R.H., an Englishman, now eighty-five years old, and fairly hale, who resides away in the forest several days canoe journey from Manáos, and whose last visit to cooler climates (England) occurred in 1847.

The general maintenance of health and energy would be enhanced by the establishment of fresh, or the improvement of existing means of transit, whereby better facilities for recreation would be afforded. Further, the welfare of European residents would undoubtedly be increased by an annual holiday trip for a few weeks, for instance, into a fresher climate, and change of scene and diet. At present, apparently, there is no definite system of holiday, so that in this respect the clerk in Pará is less fortunate than his brother at home, although he is exposed to a far less favourable climate. How far the towns on the seaboard of the northern Brazilian coast would be suitable for such a purpose, were good accommodation available, cannot be said without personal experience. Probably the change of surroundings would be insufficient, and at any rate these places are not used for health purposes to any extent either by foreigners or natives. The nearest place which seems to be suitable is the island of Barbados, which is a four-day voyage from Pará, and is not infrequently called at by the steamers on the service between New York and Pará. However, the quarantine board of the island impose a period of fourteen days quarantine—a period which seems to be fixed as a ‘useful period for all infective disorders.’* This regulation necessitates a sojourn of ten days at the quarantine station (Pelican Island) and practically cuts off the use of Barbados as a recuperating station for the Amazon region. A little more freedom in rational framing of regulations, so that some distinction were made between immunes and non-immunes, and a limitation of detention to the latter class† for six or seven days, would redound to the mutual advantage of the island, and the residents on the Amazon.

Mortality. At the present time the death rate, etc., are calculated on a supposed population of 100,000 inhabitants in Pará; it would appear that this estimate is

* No distinction from the yellow fever point of view into ‘immunes and non-immunes’ is recognized by this board, although the classification has been found useful in the United States, into which immunes are allowed free entry without a detention of a period to complete five clear days, which is exacted for ‘non-immunes.’ Again the period of fourteen days is far beyond the limit of the incubative stage of yellow fever, which at most does not extend into a seventh day. Although small-pox is apparently also feared, no particular attention appears to be paid to whether persons are vaccinated or not. During my passage up to New York the vessel was loaded with sugar in quarantine. This consisted in taking a gang of labourers on board to stevedore, and keeping them until the next day, so that if any infected and infecting condition obtained upon the vessel, these labourers were exposed as much as possible to infection. When the loading was complete the men were taken directly ashore and allowed to distribute themselves to their homes. Much red tape is also employed in the signing of ‘permits’ to land and not to land labourers. This record may be of interest to the historian who wishes to see the amount of uncommon sense which was displayed in quarantine practice at the commencement of the twentieth century.

† I should be inclined not to consider a recent convalescent as an immune until about six weeks had passed since his being taken ill; since, apparently, a sort of secondary attack may sometimes occur about a month after the original attack.

rather too large for the city alone. Dr. AMERICO CAMPOS¹ quotes the figure 29,121 as the population in 1872, in 1896 it had risen to 60,218; by estimate from these figures the population in 1900 is worked out as 75,089 for the city alone, and 101,619 for the city and suburbs and a number of neighbouring places; this last figure becomes 133,000 when another mode of reckoning is adopted. The deaths agree in number with the official figures of the interments in the city cemeteries, so that the larger figures should probably not be taken into consideration for estimating the mortality rate of the city itself. During the five years 1895 to 99² 16,346 deaths were registered in the city, which, at the supposed population of 100,000, gives an annual rate of 32.69 per thousand inhabitants. That this is much too low is probable from the accredited expansion of the city, and if we take an estimate from the above population figures of 60,000 in 1896, and 75,000 in 1900, the death rate works out at about 50 per thousand. The following gives the extreme ends of the period :—

In 1896 there were 2,492 deaths : at population of 60,000 = 42.2 per thousand.

“ “ 81,000 = 30.7 “ “

In 1899 there were 4,806 deaths “ “ 100,000 = 48. “ “

“ “ 133,000 = 36. “ “

In 1900 the distribution is :—

1st three months 1076 deaths.

2nd “ “ 1288 “

3rd “ “ 1270 “

October “ 333 “

November “ 348 “

December “ 353³ “

Total ... 4,668 “

at population of 75,000 = 62.2 per thousand.

“ “ 100,000 = 46.68 “ “

“ “ 133,000 = 35.0 “ “

Even taking the higher figures for the population, the figures are by no means satisfactory, and so far as death rate is a gauge, the general health of Pará cannot be considered in a very enviable position. Perhaps a certain allowance might be made for the deaths of recent immigrants, amongst whom there must be a considerable number of deaths from yellow fever, which would affect the death rate as an index of the health of the acclimatized and prolonged resident. Again, a certain number are accountable by deaths of rubber cutters, who have contracted their fatal ague away in

1. *Pará Medico*, Dec., 1900.

2. A. Campos. *Pará Medico*, Jan., 1901, p. 68.

3. Average from Nov. 348, and Jan. 358, there being no figures to hand for the month itself.

the forest, and come to the city to die. Out of the total of 2,364 deaths in the first six months of 1900 (*loc. cit.*), 1,042 are ascribed to infective diseases, including malaria, yellow fever, smallpox, etc., which are not separated.

In judging these figures some comparison is desirable with other places. It is not fair to make a contrast between figures obtained in tropical places and those in temperate climes. Perhaps the fairest comparison may be gained by taking the statistics of British Guiana¹ as a place where the climatic conditions are not very dissimilar, and their geographical position not remote from one another. The temperature (British Guiana) is given as varying between the limits 88.3 and 70.5; the humidity 77 to 83 in 1900. The death-rate given for 1899 is 29 per thousand, and in 1900 it fell to 26; the population estimated at 294,943. It may be remarked that yellow fever does not appear on the list, and has been effectually stamped out for some years; also smallpox only accounts for one death; tuberculosis forms the largest item with 294 deaths, and of acute diseases dysentery comes next with 85. For incidence malaria is highest, but the deaths accounted to it are not so large.

It will be seen then that the mortality rate of Parà does not compare favourably with that of British Guiana; moreover, not only is there room for improvement but improvement should be an actual possibility.

Turning to the child mortality in Parà, of which there are some figures forthcoming,² between the ages of 0-5 years for the two years 1898-9, we find 824 and 1,527 deaths respectively; more than half the deaths occurred during the dry season in each case. Out of the grand total of 2,997, infective diseases account for 749, *i.e.*; 'malaria' 545 and other diseases 204. In the light of the recent observations on the meningeal form of the fever in yellow fever in children³ the figures under the heading 'Molestias do aparelho encephalo-rachidiano e orgaos dos sentidos,' which amount to 229 may be of interest; Dr. CAMPOS says that only three children died of yellow fever during the biennial period. Measles accounted for 123, the remainder being due to smallpox, tuberculosis, whooping cough, diphtheria, and dysentery.

The percentage mortality of the total deaths of all ages is given at thirty-nine per cent. in 1898, and thirty-five per cent. in 1899.⁴

Merely as giving a suggestion of the relative numbers of deaths due to ascertained recognized specific causes, the following figures are quoted from Dr. CAMPOS' summary of the deaths in January, 1901,⁵ when for the first time some classification, under different causes, was introduced in the tables.

1. *British Guiana Medical Report* for 1900; printed for Colonial Office.

2. *Parà Medico*, pp. 59 and 60.

3. Azevedo Sodré and Couto: *Nothnagel. Sp. Path. u. Therap.* Bd. V Theil IV, Abth II, p. 160.

4. *Ibid.*, p. 34.

5. *Parà Medico*, February, 1901, p. 101.

The total deaths for the month were 358, or a proportion of 35·8 on the estimate of a 100,000 population. Some of the diagnoses given can hardly be given in English, but the style of diagnosis and its uncertainty in some of the headings may be judged of :—

Disease	Deaths
Impaludismo (malaria)	8
Cachexia palustre (malarial cachexia)	15
Febre intermittente (Intermittent fever)... ..	4
Febre palustre* (malaria)	5
Febre remittente typhoide (?)	1
Febre biliosa*	3
Febre typhica	2
Accesso pernicioso*	6
Polyneurite palustre	2
Febre amarella (yellow fever)... ..	31
Tuberculose pulmonar	23
Variola	15
Beriberi	1
Dysentaria chronica	1
Lepra	1
Angina diphtherica... ..	1
Angina croupal	1
Tetano traumatico	2
Total	122

The following remarks are derived from conversations with the local physicians and occasional visits to the general hospitals, etc. :—

Malaria is dealt with elsewhere so that here it may be noted only that the determination of the presence of the malarial parasite, is practically a perfectly unused mode of diagnosis. A good many of the cases are imported from the islands and other rubber districts. Bronchitis when diagnosed is often ascribed to malaria.

Tuberculosis is one of the chief scourges of the place, as the above figures indicate, the small dark houses, the universal and free spitting everywhere and anywhere, and the fine siliceous dust in dry weather may be regarded as contributive factors. I was informed that certain old sage negro women were in the habit of applying sputum to the breasts of mothers as a means of child murder.

Variola ('Bexiga') is also one of the banes of the place. Much effort has been made to secure vaccination, but the practical difficulties are very great and only a small proportion are thus protected;—revaccination is probably still more rare. An isolation

The headings marked * are sometimes synonymous with yellow fever.

hospital (Sao Sebastian) was opened on February 14, 1900; from this date to the end of the year no less than five hundred and eighty-five patients were admitted. Of these one hundred and seventy-three died; but it is noted¹ that many of the deaths were due to other causes than variola (*e.g.*, 'malaria, beriberi and cardiac, and renal complications'), and during our stay we came to learn that one of the devoted nursing sisters at this hospital succumbed to an attack of yellow fever. Varicella apparently also occurs.

Measles ('sarampo') is not uncommon, and epidemics occasionally occur; during our stay we saw a few cases. Pertussis is also not uncommon. Ordinary and severe 'colds' in the 'head' are frequent.

Beriberi occurs, but is not very common; a few cases were seen.

Yellow Fever ('Febre amarella') was introduced into Pará² from Pernambuco by means of the vessels 'Pollux,' which arrived from the fever-stricken port on January 24, 1850, and the 'Pernambucana,' which arrived from the same port a few days later. Two sailors of the former died on the last day of the month, and on the next day three sailors from the latter died; no further deaths are recorded during February which is of interest; in March there were forty-one, and April two hundred and sixty-nine. During the year three-quarters of the population were attacked, which suggests that fatal epidemics in previous years were due to other causes, as variola, etc. Thus a total of twelve thousand persons out of the total population, estimated at sixteen thousand; the deaths ascribed to the yellow fever were five hundred and six from January to July, deaths due to other causes being three hundred and four; altogether corresponding to an annual rate of one hundred and one per thousand. Presumably the deaths due to yellow fever were more numerous than was admitted, owing to concealment, so as to obtain interment in churches (of which there were one hundred and eleven during the period) and consecrated ground.

Soure and other places in the neighbourhood apparently were infected very soon, but no doubt from the slight amount of commerce up the Amazon the disease was not carried along this waterway till a much later date. Manáos was severely stricken during recent years, chiefly, no doubt, through the importation of numbers of non-immune labourers. From accounts it appears that the infection has been spread up as far as Iquitos, two thousand miles or so up the river.

Apparently the fever has remained to stay in Pará ever since. The local boast that this and other bad illnesses are imported and not national is not a sufficient excuse for not stamping it out.

There are some indications of the prevalence of the fever in the city during recent months by the entries of patients and the death roll at the isolation hospital,

1. *Pará Medico*, I, p. 21

2. A. Vienna, *Pará Medico*, I, p. 35.

'Domingos Freire.' This was opened on the 29th of April 1900, for the reception of patients.

	Month		Admissions	Deaths
1900.	To end of May	...	83	33
	„ June	...	94	42
	„ July	...	87	42
	„ August	...	85	30
	„ September	...	56	20
	„ October	...	34	12
	„ November	...	31	9
	„ December	...	44	11
1901.	„ January	...	51	21
	„ February	...	45	10
	„ March	...	29	5

Amongst these cases are a small proportion due to other diseases, but the majority are due to yellow fever. The figures, however, are merely an indication of the amount of the disease that was prevalent at the time, for there is no compulsory isolation, thus many cases remain distributed at their own homes ; most of those that found their way into the general hospitals were transferred, but not always ; lastly, there are a number of cases which are not recognized, or are called by other names.

Leprosy cases, when recognized, are sent to an isolation hospital out in the forest at the back of the S. Isabel region. The patients seen were mostly rather advanced examples of the disease, and were one hundred and eight in number at the time of my visit. The number corresponds to 0.5 per thousand of the population of the whole state. The Rio Tapajos by repute is the chief centre of the disease ; probably not more fish is eaten there than in other riverain places.

Venereal complaints are common. Gonorrhoea is very frequent, and I was told that the majority of the female patients who applied for treatment at the general hospital were either suffering from some form of this or from phthisis. Amongst the patients at the yellow fever hospital we did not notice much syphilis, or signs thereof.

Diphtheria is quite rare ; an occasional case occurs, but it appears that the disease does not tend to spread. Whether this is due to an absence of the conditions which cause its spread in other climes, or whether it exists in a modified and unrecognized form of tonsillitis, cannot be said. The children on their way to school may be seen carrying their little bottle of ink and pens, slates are probably too expensive an exotic luxury.

Scarlatina is another disease which is said to be practically unknown ; again, either the climate, etc., is not suitable for the extra-human life of the causative agent, or else the conditions necessary for its spread from individuals are absent.

Typhoid Fever and Dysentery are also very uncommonly met with or diagnosed as a local product. Such water supply as there is seems to be fairly well protected from contamination at the source.

Yaws and Lobar Pneumonia are not recognized.

Tetanus is not uncommon.

Rabies is said to occur now and again. Lately steps have been taken to undertake the preparation of spinal cords for Pasteurian treatment. Hitherto, when required, the material has been imported. Curiously enough, the more effective and economical step of clearing out the more or less ownerless and scavenging dogs has not been taken. Numbers of dogs, usually in a disgusting state of mange, covered with ticks, and their feet full of jiggers (*Sarcopsylla*), are seen about the town, which might well be destroyed, and would be calculated to make the disease common, when it is introduced.

(I understand that Manáos was once cleared of stray dogs by the cruel method of transporting them over the river to an island).

For practical purposes the acute diseases at Pará, which are the most dire for the place and region, are malarial fevers for which a careful survey of the *Anopheles* breeding-grounds is desirable; so far as they were seen, they ought to be easily dealt with in the precincts of the city. Smallpox which is already being attacked by vaccination and isolation. Tuberculosis, which requires the introduction of more hygienic personal habits amongst the people. Yellow fever, which requires the careful and early recognition and notification of the cases, their early and compulsory isolation, and the destruction of breeding-grounds (casual waters, cesspools, rain gutters, etc.), for mosquitoes about the houses. Filarial disease has not been mentioned above; a few cases are seen of obvious lymphscrotum, and a few cases of elephantiasis about the town. The protection of the general hospitals by means of permanent 'bars' in the windows; doorways to keep out mosquitoes, and so prevent these places from being a sort of filaria exchange is all that need be said, except that it would be advisable for persons to avoid having infected persons living in the houses. It may be noted that one of the mosquitoes caught in one of the hotels was found to contain an embryo filaria.

It seems that the undertaking of any of these points would be more profitable than a considerable outlay against more or less hypothetical rabies, and conduce to the improvement of the local sanitary condition.

It remains to say a few words concerning the hygienic steps taken by human interference apart from the natural conditions which obtain.

Water Supply. The supply of water is bad, from the points of view of quantity, quality, and the possibility of contamination. For more than two years the water has not been sufficient in quantity, partly owing to the inadequate size of the mains and the pumps; thus in one house in the city it was not possible to get a shower

bath on the ground floor after about eight a.m. ; at the hospital, Domingos Freire, no water came through the pipes after eleven a.m. In many houses private pumps and cisterns were arranged so as to ensure a supply through the day. As regards quality, it may be noted that the water as it flows from the tap is frequently very full of brown sediment, so that it is quite opaque ; dead flies, mosquitoes, and their larvae have also been encountered in the water flowing directly from the tap. When the water supply ceases and the tap is turned there is strong insuction of air giving the possibility of the introduction of other things. The amount of water is given as over 1,000,000 litres per diem,¹ that is ten litres per head per day. Especially in outlying districts local wells or springs are used. The community generally is wasteful, and much water finds its way in watering gardens, etc. Public standpipes are provided about the city so that the poor can obtain water free of charge ; in houses where it is laid on it is taxed by meters. The gathering ground is a charming collection of springs at Utinga well away from the city beyond the Marco da Legua. We were promised an official visit of inspection of the new works that are being put in, but, for some unaccountable reason, it never took place. Except one stream, passing down in close proximity to the pumping station, and in which people bathe, wash clothes, and which is crossed by a public footpath, and liable to pollution from several houses in its proximity, and of which we were unable to determine the eventual fate of the water, the springs and streams seem well sheltered from pollution. New works are being pushed forward, including large mains of nearly one metre diameter ; naturally this is slow work, and the temporary laying of a small subsidiary main for immediate purposes was not adopted, so that the water supply is not likely to be very adequate for some time longer. It may be mentioned, in passing, that these large pipes for the mains have been lying about in most indescribable filth in the roadways, etc., for some time, others have been employed as dormitories, so that when the new service is instituted persons should be more than ordinarily careful about the filtration and the boiling (if the servants are sufficiently reliable) of water for consumption.

Sewerage. Some part of the city is supplied with drains, mostly at any rate the mains are old brick constructions, and probably not free from faults. The amount of water available for flushing out is quite insufficient, though the rivers afford an unlimited available quantity. It seems hardly worth considering alterations until there is sufficient water available for efficient water carriage. Probably the major part is served by cesspools ; these are such that in many cases the watery constituents are said to filter away into the sand, leaving a very slight amount of solid residue ; those which I saw, however, were full of water, and formed an abundant source of the *C. fatigans* mosquito.

1. *The State of Para*, 1893, p. 113.

For surface drainage many of the outlying streets are unpaved, and consequently are liable to be full of puddles ; here a system of excellent side gutters of cement has been put in, and thus much of the rain-water is rapidly carried off. These are being extended and the lie of the land is sufficient to give gradients in most places ; naturally some of the older gutters are more or less obstructed in parts by the rubbish, and hosts of mosquito larvae are to be found in the retained water ; but on the whole they are kept clear, a condition which is aided by the tremendous flushing which occurs during a rain shower. The sandy nature of the soil assists greatly in the natural destruction of gnat-breeding puddles.

The rain gutters which take off the water from the roofs requires serious consideration if the city is to be freed from mosquitoes. They are frequently fixed so as to be almost horizontal, well supplied with bends, corners, and dead-ends, so that there is every opportunity for retaining water from the constructional point of view ; more than this, a walk along the streets will show that many are obstructed with growing plants or trees. There is quite an opening for justifiable municipal legislation to deal with the necessity of keeping all such gutters free from obstruction. Also when they are put up, since they are frequently inaccessible, or only accessible with difficulty, they should originally be put up with a considerable gradient, so that they will tend to clear themselves out and give no chance of stagnation. Moreover, downward pipes should be provided at acute turns. The water gutter is a serious question in Parà since the almost daily showers in the dry season tends to keep them constantly filled with water if there is obstruction.

Rubbish destruction. In their praiseworthy efforts to improve the sanitation of the city, a system of collection and destruction of household rubbish has been instituted by the authorities. In the evenings householders deposit the rubbish of the day in the gutter opposite their houses, and if these are on the track pursued by the collecting carts, as the principal streets of the city, it is collected and carried away to the furnaces. Recently some new furnaces have been erected, which should be capable of doing the work in an economical manner ; unfortunately, however, the nature of the rubbish to be destroyed does not seem to have been adequately studied before the furnaces were designed, so that on trial they were not found entirely satisfactory. In the more outlying districts the rubbish remains and the effluvia are unpleasant. The mode of cartage is clumsy, and would be much facilitated by the use of some of the tramway tracks and extensions thereof. The sweepings from the stables of the tramway company (Compania Urbana) are carried out beyond the city to the grass fodder growing district at Sacramento ; in the neighbouring forest are some choice spots, where the carcasses of dead mules and oxen are dumped from time to time ; the latter are to be dealt with by cremation at the destructor furnaces.

Living houses. It cannot be said that the general plan of the houses is suited to the climate ; even in comparatively good houses there is absence of verandahs and good

arrangement for ventilation. The poorer class mostly live in dark dens in the city not arranged for through ventilation ; in the outskirts there are some terraces of cottage quarters, though as one proceeds out detached palm-thatched huts are the rule. In the latter the people keeps things clean and tidy, with the exception of free expectoration on the dried mud floors ; but more within the city the quarters are often very filthy. As a race the Brazilian is of cleanly habit, so far as personal washing and linen go. The almost universal use of the 'rede' (a kind of hammock) instead of bed for sleeping may conduce to the reputed absence of bugs and the comparative rarity of fleas, although these must be constantly imported by the indigent immigrant from the slums of Portuguese, Spanish, and Italian towns.

Hospitals. Lastly, a word must be said about the hospitals. A large general hospital, Santa Casa de Misericordia, has been built in the Umarizal quarter of the city. The large wards seem airy, well kept, and clean. The basement is also used for wards, and though well kept the conditions of light and air are not so good. Copious water supply is ensured by pumping up into large storage tanks. There is also an elaborately decorated and furnished committee room, the expense of which might well have been foregone to admit of the introduction of more immediate necessities. The chief fault is one of omission, for there is no provision for keeping out mosquitoes ; large numbers of blood filled mosquitoes are to be found any day in the dark corners ; there can be no question that all window spaces and doorways should be protected with permanent wire gauze nettings. In a hospital it is not possible or advisable to have individual nettings for each patient ; with the movements a certain number of gnats would be almost certain to effect an entrance, these could easily be dealt with by inaugurating a brigade of convalescents armed with small 'butterfly nets' ; perhaps, in certain instances, such as malarial and filarial cases of diseases, the patients might be kept in a specially guarded ward ; the same would be done in yellow fever suspects. At the same time all breeding places for mosquitoes in the neighbourhood ought to be kept under survey. With such improvements in many ways the hospital may well bid as a model hospital for tropical cities.

Also under state or municipal control are the isolation hospitals for yellow fever and smallpox ; the latter was not seen, and the former is deserving of praise in its cleanliness and brightness. Better water supply arrangements are urgently needed, and what has been said of making the Santa Casa mosquito proof, can only be repeated here. The nursing is done by the sisters of an Italian sisterhood (Sta Anna) in all these hospitals ; the people, perhaps, hardly realize how much they are indebted to the devotion and care of these good women, who come out at considerable personal risk to minister to the sick, and not a few have added to the death roll, chiefly of yellow fever. The isolation for leprosy (Asilo dos Alienados) is situated away in the forest, and consists of a number of huts and houses with a common refectory, etc. There is also an asylum for lunatics out at the Marco da Legua ; this was not visited,

it is said that there was a severe visitation of beriberi at one time. Besides these there are other hospitals such as the 'Portuguese' and the Ordem III of S. Francisco.

Lately a quarantine station has been put in working order at the delightful little island of Tatuoca, about twenty miles down river from Parà ; it is supplied with disinfecting apparatus and accommodation for patients and detention of suspects. Before this was opened the nearest quarantine station was that of Ilha Grande, at a distance of about two thousand miles. Ghastly as it may seem vessels have made this journey at the bidding of the panic stricken authorities ; now that this has been rectified by energetic pressure no more need be said on the matter.

MOSQUITOES

Remarks on the hygiene of a place in the tropics would not be complete without some reference to dealing with the mosquitoes. In some houses visited, they are so numerous that even poorer people make use of mosquito nets for sleeping under ; this, however, is not general. It appears that old inhabitants and natives are by no means insusceptible to the annoyance of the insects, still on the whole they do not trouble to protect themselves ; nor are any steps taken to treat the breeding-places of the insects.

English residents with whom I have spoken say ' what is the use of my dealing with the precincts of my house unless my neighbours do the same, for I shall get so many gnats from next door that my own endeavours will be useless for my own comfort.' This is, no doubt, true to some extent, and really forms the key to dealing with the mosquito question, namely, that to be of real use organized and combined and continued attacks upon the insects must be made. Dilettante destruction, or temporary energy, here and there, will probably never be of any real service. There is much that might be done in Parà in this respect, and probably the attack on breeding-places is the most practical method of dealing with the matter. The construction of the houses is not very favourable for fixing up permanent wire gauzes, such as one sees in the United States, and which are being introduced into other regions.

Besides the cesspools and rain gutters, which have been mentioned elsewhere, the carelessness of people leaves all sorts of neglected collections of water about the houses, wash tubs, odd tins, etc. (In Cuba, the Americans deal with these by fining householders for having unnecessary and unguarded water accumulations on their premises).

The flushing tanks of water-closets which are in constant use do not seem to be great breeding-grounds, unless they are left unused ; still there is no harm in having them covered with gauze.

These sites, with possibly the main sewers which are constantly fed from gutters, etc., filled with larvae, seem to be the chief breeding-places about the paved part of the town ; the gardens, often with fountains and circular water troughs round the plants for keeping off the destructive Sauba ant, also require attention for they form a fertile source of *S. fasciata*. In the unpaved parts, naturally, there are many odd pools from time to time, those of natural origin from the configuration of the ground, and those due to obstructions in the roadside channels, especially the unconcreted ones. The pools in which *Anopheles* larvae were found, as mentioned elsewhere, are eminently adapted for treatment by filling up on account of their small and shallow nature ; a complete survey of these is required. Living specimens of the larvae and adults of the *Anopheles* were shewn to many of the local medical men and others.

Experiments were intended, but never carried out owing to stress of other things, to try the culicidal effect of poisonous plants in the local flora. About Pará are numerous examples of the Solanaceae (for instance, the 'jurubeba' *Solanum grandiflorum*), and if means like the leaves or other parts of common local plants will act, when placed in casual waters from which drinking supplies are not obtained, by killing contained mosquito larvae, a simple and economical warfare can be waged on the insects without much exertion.

A few words may be said about mosquitoes on the ships about Pará. It is said by captains that mosquitoes only commence to come on board when the lighters which are used for the discharge of the cargo are brought alongside. Seeing that the anchorage for the large ships is some two miles below the city, and the lighters lying about the neighbourhood of the city are brought to wharfsides for discharge, they are a means of bringing city mosquitoes to the ships. On examination, several of the large barges (which are covered with a rainproof metal cover) were found to harbour a certain number of the insects, both *S. fasciata* and *C. fatigans* were found in the adult condition and a few larvae of the former in the bilge water ; but these lighters had recently been cleaned up and painted ; open lighters are also used, these were seen to contain abundant rain water and tar and oily material from the coal for which they are used ; no larvae were found in them.

During my trip up to Manáos, indiscriminate collection of all and any mosquitoes that could be found on board was made. Although a number were collected it was not until after anchoring at Manáos and the lighters came alongside that the species *S. fasciata* was taken. It appears that the method of discharge by means of lighters may be almost as risky for the importation of mosquitoes infected in the city, as if the vessel was actually brought to the wharfside. The only recommendation that seems likely to be effectual would be to fumigate the lighters some time before they were brought alongside by burning sulphur within them.

A certain amount of breeding of mosquitoes may occur upon the vessels; thus one captain told me that he once discovered larvae in the water tanks. The telegraph ship which has been many years upon the Amazon was examined; very many *S. fasciatus* and some other gnats were seen and taken. Besides it was found that there were abundant larvae of *S. fasciatus* in the bilge water; some permanganate of potash had been put down with a view of killing them, but the quantity was probably much too small and most of it soon decomposed; the trial of a small quantity of kerosene oil was suggested as a more likely means. In vessels such as this which remains long on the river as well as those which are only a short time, there ought to be provision of netting to fit portholes, doorways, lights, etc., so that at any rate a certain amount of protection and comfort might be obtained.

IX. ODD NOTES

A. ON THE ETIOLOGY AND TREATMENT OF A SKIN ERUPTION KNOWN AS 'PRICKLY HEAT'

A form of irritating acute skin eruption is widely spread in Parà ; by the English-speaking folk it is called 'Prickly Heat,' and so far as the description goes it seems to be identical with what is commonly understood by the term.

The chief points to be dealt with at present are :—

1. Its infective nature.
2. The living organism associated with it.
3. The mode of treatment and cure.

The infective nature could clearly be traced by the course followed upon myself. On the ulnar aspect of each wrist, for weeks after arrival at Parà, I was troubled by what I took to be an unusually swollen and long-lasting gnat bite. My attention was especially attracted to that on the right wrist a few days later by the appearance of an irritating patch of redness, with small vesicles about the middle of the forearm, and also at the same time a similar condition had spread about the neighbourhood of the original papule. I found that the sites corresponded with the points of contact of my wrist and forearm with the edge of the table during the use of the microscope ; and it appeared clear that the patch on the forearm was due to implantation of the causative material from the wrist to the table, and so to the forearm. Not long afterwards a patch appeared on the front of the lower part of the chest, which I found was a common point of contact for the patch on the forearm. Later observation shewed that direct infection by local contact could occur as from a spot on one side of the bend of the elbow or fold of the axilla to a corresponding contact point on the other side. It seemed probable also that a certain amount of spreading might be due to rubbing or scratching 'without antiseptic precautions.' To cut matters short, before the condition was properly dealt with it had spread more or less universally. It may be added that there was only a slight amount of spreading in the near neighbourhood of the initial lesion on the left hand.

My late helper and colleague, WALTER MYERS, also suffered in the same way about the same period, but except that he was inclined to attribute the initial lesion to a gnat bite I am not aware of the course of the distribution.

Although suspicion attached the original inoculation to the bite of a gnat, which if this were the case, would almost certainly have been *Stegomyia fasciata*, this was by

no means proved. It may quite well have been due to handling the yellow fever patients, many of whom were affected. This is a point which can only be determined by careful watching in an individual who has never before been affected on a first visit to tropical regions.

The lesions seen at the very earliest consist of a patch of inflammatory reddening with a tiny vesicle at its middle; the vesicle enlarges, but generally remains quite small; the contained fluid is at first clear, but later may become turbid and finally purulent if it persists and does not disappear. Where there has been local spreading a considerable area of skin may be reddened and scattered about upon it are numbers of the vesicles; these are generally more minute than when a single isolated vesicle develops. In distribution they are between the hair follicles, and presumably are due to the involvement of the sweat glands. Occasionally, however, they may be close to the hair follicles, and these sometimes appear to get involved. Presumably, at the moment when the vesicle becomes tense, a sharp, intense sensation is produced, and the inclination to rub or scratch is very great. Rubbing or scratching, however, rather tend to increase the irritation. After a time the local condition subsides spontaneously, but it may reappear at the same site on a future occasion; meanwhile other areas are in the acute stage.

Microscopic contents of the vesicles. With the aid of some squeezing and a very fine capillary tube, the contents of the vesicles can be removed and examined. When the vesicle is not too far advanced the fluid is clear, and is generally found to contain a few red blood corpuscles but no leucocytes; at a later stage the leucocytes appear in greater or less numbers and give the purulent character. What attracts the attention at the early stage, before the advent of any or at any rate many leucocytes, is a number of small bodies endowed with active amoeboid movement. Their protoplasm is more refractile than that of the 'polynuclear' leucocyte, and contains a small number of granules of a highly refringent character. The changes in shape of these amoeboid bodies are rapid without artificial heating of the slide at the ordinary afternoon temperature (27° - 30° C), the pseudopodia being generally comparatively blunt and rounded. The accompanying sketches shew some changes in shape, which occurred within the space of a minute or two (Plate VIII, Fig. 3).

When the suppurative change has commenced large numbers of polynuclear leucocytes are to be seen, either entire or more or less disintegrated; micrococci in pairs or in groups are also present in variable, generally small, numbers. Active amoebae, however, are then rarely found, but there are some globular bodies which would correspond in size to and which are possibly of the nature of encysted amoebae.

The abundance of the amoeboid bodies at the earlier stages and the absence of micrococci or other bacteria at this time make it probable that the formation of the lesion is concerned with the presence of the amoebae, the later invasion and suppuration, when it occurs, being caused by the micrococci or other bacteria.

In regard to the pathology of the condition, the circumferential redness is suggestive of some chemical as well as of a mere mechanical action ; at the same time there does not appear to be any marked effect upon general health.

These observations were made upon the contents of vesicles occurring upon my colleague and myself.

Treatment. It has already been observed that the lesions in one place resolve spontaneously, whether the active agent merely lies latent or actually dies, so that reinfection is necessary for the same area to be reinfected cannot be answered.

The adult natives do not appear to be troubled, but the small babies are often seen with what appears to be an identical condition. At the same time, individual immunity is not always acquired by long residence, as I met with a gentleman who had been about Parà for more than ten years, and who said he had never been so troubled with prickly heat before, and certainly he was pretty well covered with the lesions.

A considerable number of different applications were put to the trial. Those which were found useless may first be mentioned and disposed of. The 'palliative' applications of toilet powder, of boracic acid in powder or solution, lysol (one per cent.), carbolic acid (five per cent.), alcohol, permanganate of potash, sulphurated potash (five to ten per cent., which is quite effective for dhobie itch), were found to be perfectly useless ; they neither relieved the itching nor did they cure the condition. Numerous shower baths, whether followed or not by a sponging with weak permanganate of potash and spontaneous drying, likewise did no good.

The two applications which were found of service were iodine and corrosive sublimate. The former was used in the form of tincture diluted with spirit so as just to stain the skin well ; this has the disadvantage of staining the skin and also marking any starched linen temporarily. The corrosive sublimate was used in solution of about 1 : 500 to 1 : 1000 in spirit and water, in water alone or in water glycerine and spirit (the idea of the glycerine being to prevent irritation by the drug). The first of these seemed to be the best, though its superiority was not great over the others, for plain aqueous solution acts perfectly well. The mode of application was to rub well with a pledget of cotton-wool well wetted with the solution ; the next day the red inflamed areas have disappeared, and the sites of the more deeply infected follicles have a brownish colour instead of a bright pink, and on rubbing they are not so irritable. Two or three applications may be required to get rid of the deep infections, and it seemed more satisfactory to go over considerable areas at a time. The most deeply affected follicles are the most difficult to eradicate entirely, and some were treated by expression after the manner that the old housewife extracts a wasp sting by means of a key barrel, with the pressure of the end of a small glass tube, in which a small quantity of the antiseptic solution is held by capillarity. This, which is an effective mode of evacuating ordinary suppurating follicles also, is not altogether a pleasant

proceeding, and a doctor might be advised to try on himself before he subjects his patients to it. Refractory follicles may be touched with strong iodine tincture.

Corrosive sublimate has the disadvantage of being unpleasantly poisonous to leave about in lay hands for promiscuous application, so that some less dangerous material or form of the material would be advisable for practical purposes. As a suggestion, which gives promise of being well worthy of trial, I may mention the anti-septic soaps which are used for obstetric and other purposes, or more precisely those which contain mercurial salts (generally the iodide, I believe). A cake of such a soap would not be so objectionable on the score of its poisonous properties. Naturally, for portability, a cake of soap does not compare with a few tabloids of Hydrarg. perchlor : a small portion of one of which will do a good deal. Enquiry may reveal some more preferable protoplasmic poison ; formalin and salts of copper were not tried.

It is not without interest to observe that the ordinary suppurating follicle with staphylococci is readily cut short, with carbolic acid (*e.g.*, sat. sol.), whilst the prickly heat is not affected as would be probable if the skin staphylococci were the cause.

Summary. The condition of 'Prickly Heat' studied is infectious by contact ; it is associated with the presence of active amoeboid bodies ; it is curable by means of applications of corrosive sublimate, and, probably, other protoplasmic poisons.

B. DREPANIDIUM IN THE TOAD

All the smaller toads which we examined at Parà (*i.e.*, about the size of the ordinary English toad) were found infested with a species of *Drepanidium*. Two main forms of blood parasite were found, in the red-blood corpuscles one corresponding to the ordinary drepanidium and the other to LABBE's *Dactylosoma*. The former seemed to multiply chiefly, if not solely, in the internal organs (liver), the latter inside the circulating red-blood corpuscle. Once inside a red-blood corpuscle, it appeared that the drepanidium form did not leave it within the body of the toad ; for by fixing large quantities of blood from the heart immediately with sublimated saline solution and centrifugalizing we never succeeded in discovering free drepanidia ; on the other hand, if the blood was examined without a fixative agent or centrifugalized with plain citrated saline, after a short while no endocorpuscular drepanidia could be found, all having become free. This is suggestive that the drepanidium may be destined for a life in a second host. This host is, in all probability, to be found in the ticks (*Ixodes*), with which almost all the toads found were infested. Examination of the contents of the ticks shewed curious cysts, evidently different from the curious nuclei of the tick's economy, varying in size up to about 60 μ . It was noted that the movements of free drepanidia in the fluids from the tick's stomach were much more active than in the toad's blood ; appearances suggestive of conjugation were also seen. Owing to our yellow fever work the observations were necessarily fragmentary ; moreover,

we could not obtain uninfected toads for trial of experimental infections. Further details may be forthcoming from the examination of the prepared material, which is to be examined. The suggestion is that the drepanidium form is the gamete which completes its development in the arachnoid host, whilst the dactylosoma is the endocorporeal permanent parasite of the toad, they were not seen to leave the corpuscle in material either from tick or toad.

An observation which may be of interest in the economy of tickborne diseases, for instance, in Texas cattle fever in which the young ticks are capable of carrying the infecting agent, is that a tick which had been left in a box laid eggs which had hatched out during my illness. The young ticks had eaten nearly all of the contained blood and organs of their mother. *If the tick stages of a parasite (as drepanidium in this case) were alive in the mother, this would form a ready mode of securing the continuity of the existence of the parasite; this would be especially important for the parasite in the case of a tickborne disease, for ticks usually remain upon a single host, and therefore would not be calculated to spread a disease from animal to animal.*

C. A. TRYPANOSOME

A small bat (*Phyllostoma*) which could not be examined at once was placed in a gauze cage, and with it a specimen of *S. fasciata*. The next day the bat was dead and the mosquito full of fresh blood. This blood contained abundant trypanosomes, whose shape is quite different from the usual ones in rats, Nagana, etc. Too much *post-mortem* change had occurred in the bat for satisfactory examination, but there were some structures which suggested altered trypanosomes; one knows from Nagana how soon after death of the host changes ensue in the trypanosomes. Other bats were obtained but there was never leisure to examine them until death had occurred. Although often flagellates, coccidia like bodies, etc., were found from time to time in the eighty mosquitoes which were dissected, this was the only time that trypanosomes were found.

OTHER ANIMALS

Blood examinations were made on a number of different animals. Only one out of about a dozen lizards (green ones and brown ones) showed endo-globular parasites. One small brown bird of the pigeon tribe was found full of *Halteridium*. A toucan and an agouti were negative.

In the island of Marajo, about the cattle ranches, horses, dogs, etc., are said to suffer from a disease, consisting of wasting and paralytic symptoms, called 'quebra brunda.' It occurs during the dry season, and by local repute the capibára (*capybarus*) are said to sicken when or before the disease is prevalent. We saw many cattle with abundant ticks; these were in a meagre and wasted state of health.

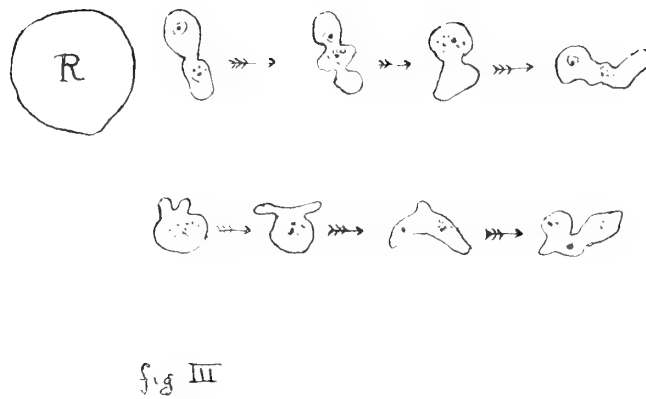
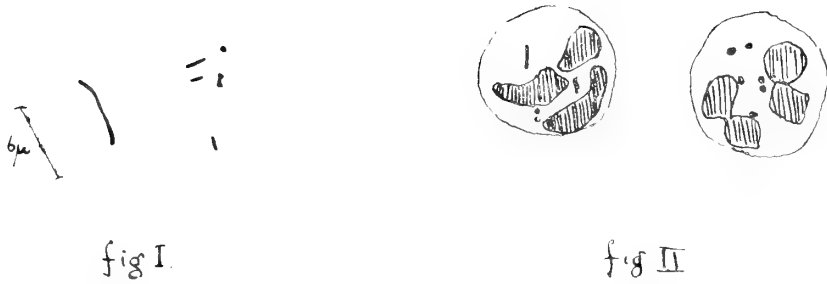


FIG. 1.—Group of 'small bacilli' from blood of 'typical bite.'

FIG. 2.—Two 'polynuclear' leucocytes from a preparation of a bite received at night about thirty hours before (mosquito—*C. fatigans*).

FIG. 3.—Sketches to show the changes in shape of two amoeboid bodies in the fluid from a young 'prickly heat' vesicle.



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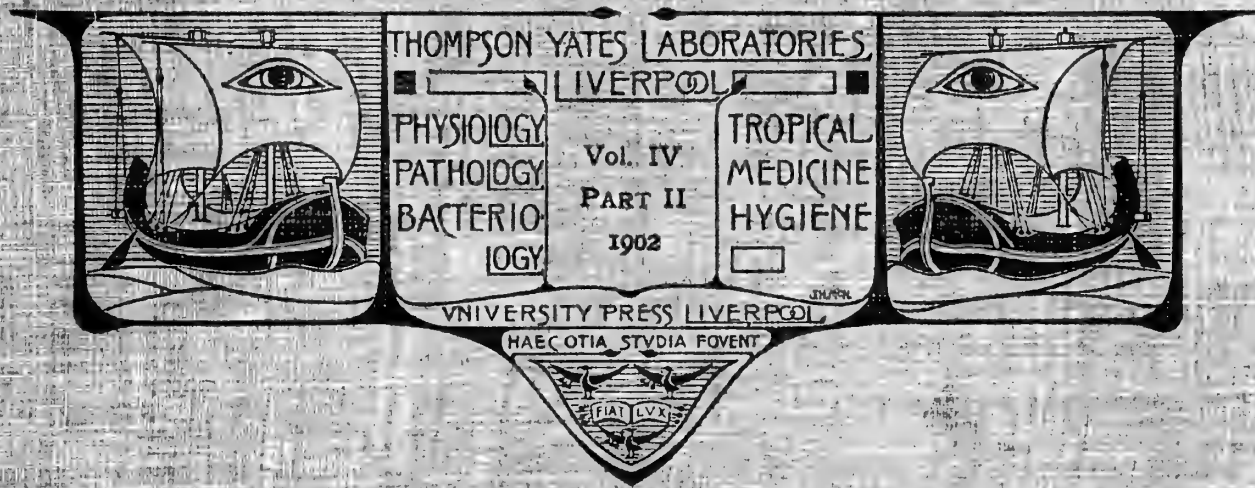
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